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THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF CERTAIN PLANTS

W. L. POWERS

Oregon Agricultural Experiment Station[1]

Received for publication August 25, 1926

A study of the effect of hydrogen-ion concentration on the growth of certain plants was initiated at a time when statements were to be found in texts to the effect that legumes grow best in a slightly alkaline medium. Bryan (1) and others claim to have obtained results which seem to show that a slightly alkaline reaction is most favorable for the growth of certain legumes and other plants. Observations of alfalfa flourishing on land regarded as basic or slightly alkaline have caused apprehension as to whether optimum growth secured in slightly acid water cultures could be duplicated with plants growing in the soil.

Attention was called to the effect of hydrogen-ion concentration on the growth of alfalfa in connection with studies on sulfur when used as a fertilizer, and these studies have been extended to certain other plants of interest in connection with the development of agriculture for the acid soil areas of the North Pacific Coast region.

The methods used in these studies included growing plants in culture solutions and soil pots of known reaction, and determinations of hydrogen-ion concentration in cropped and uncropped field soils. The culture solutions were made up to a total osmotic concentration of approximately the equivalent of 1 atmosphere. The salts used were bi-potassium phosphate, potassium acid phosphate, calcium nitrate, calcium sulfate, and magnesium sulfate. In certain cases a saturated solution of calcium sulfate was employed. To increase the buffer action of the solution, 300 p.p.m. potassium-acid-thalate was added. Molal solutions of other salts were prepared and used in amounts up to 6 cc. per liter of culture solution. The different proportions of the acid and of basic potassium phosphates were used to adjust the reaction at the beginning of the experiment. Various degrees of acidity were provided for different sets of culture jars.

The initial reaction was determined on the Hildebrand hydrogen electrode.

---

[1] These studies have been made partly in coöperation with graduate students at Oregon Agricultural College; namely, Mr. Charles Hartmann, Jr., who coöperated in part of the alfalfa experiment; Herman McCormick, with the alsike clover trial; Carl Faulhaber with the vetch trial, and Arthur Albin with mint. Rhododendrons were also used in related studies.

Released with approval of the Director, Oregon Experiment Station. Read before Western Society of Soil Science, Mills College, Cal., June 1926.

Subsequent determinations were colorimetric, the reaction being adjusted where necessary by the use of 0.2 *N* sulfuric acic, or 0.2 *N* potassium hydroxide. In the case of legumes, nitrates were nearly eliminated from the culture solution. It was found necessary to provide about 20 p.p.m. to carry the young seedlings until such a time as the bacteria and nodules could develop. The growth period was 4 to 10 weeks. With legumes, observations were made on the effect of reaction on the total growth of plants and on the development of nodules.

TABLE 1

Relative yields of plants grown in solutions of different pH values

CROP	pH VALUE	NUMBER OF PLANTS	AVERAGE DRY WEIGHT OF TOPS	AVERAGE TOTAL WEIGHT OF PLANT
			gm.	*gm.*
Alfalfa	5.2	59	0.206	0.342
	6.0	54	0.232	0.366
	7.2	57	0.128	0.166
Check	5.8	26	0.349	0.464
		NUMBER OF NODULES	TOTAL WEIGHT OF TOPS	WEIGHT OF WHOLE PLANT
			gm.	*gm.*
Hungarian Vetch (30 plants each culture)	4.5	...	0.48	1.02
	5.3	340	1.70	2.90
	6.3	5	1.55	2.81
Check	5.3	570	3.30	4.10
		NUMBER OF PLANTS		AVERAGE WEIGHT OF ENTIRE PLANT
				gm.
Mint	4.0	7	......	1.75
	5.0	7	......	1.63
	6.0	7	......	2.25
	7.0	7	......	1.72
		NUMBER OF PLANTS	AVERAGE DRY WEIGHT	NODULES COMPARED WITH pH 6.00
			gm.	*per cent*
Alsike Clover	4.8	24	1.081	5
	5.5	30	1.446	80
	6.0	24	1.464	100

Seedlings were sprouted in pure quartz sand and transplanted (in the case of legumes, when the fourth leaf appeared) into from 1 to 4 gallons of culture solutions, except with rhododendrons, where the plants, starting with very small wild seedlings, were grown in 2-quart jars.

A general summary of results of these experiments shows that all plants have grown best in a slightly acid medium. The maximum development was secured with alfalfa at a pH of 5.6 to 6.0. Hungarian vetch gave the best growth at pH 5.3, and spearmint at pH 6.0. The optimum reaction for alsike clover appeared to be between pH 5.5 and 6.0 (plate 1). The experiments with alfalfa, vetch, and mint were repeated. In the case of alfalfa the experiment was repeated at different seasons, and in different climates. The largest number of nodules was developed in culture solutions kept at pH 5.8 to 7.0, but the largest

TABLE 2

Hydrogen-ion concentration of soil pots

FALLOW	FEBRUARY 15	MARCH 10	MARCH 25	APRIL 7	APRIL 21	MAY 5
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
Check	6.8	6.2	6.2	5.7	5.6	6.1
S & Ca	6.6	6.6	5.7	5.5	5.4	6.7
S	6.6	6.4	5.6	5.5	5.9	6.3
Gypsum	6.8	6.8	6.0	5.6	5.9	6.7
K_2SO_4	6.8	6.8	6.0	5.4	6.0	6.5
CaO	7.0	6.4	6.2	5.8	6.0	6.6
Check	6.9	6.4	5.2	6.0	5.8	5.8
$(NH_4)_2SO_4$	6.9	6.3	6.0	5.7	5.6	5.9
$MgSO_4$	6.9	6.3	6.2	5.9	6.0	6.1
CaO	7.1	6.4	6.0	5.8	6.3	6.4
S & CaO	6.9	6.4	6.0	5.7	6.3	6.3
S	6.8	5.8	5.9	5.8	5.8	5.6
K_2SO_4	6.7	5.7	5.6	5.8	5.8	5.6
S—200 pounds	6.3	5.2	5.2	4.6	4.5	4.4
S—500 pounds	6.0	5.1	5.0	4.4	4.4	4.2
Gypsum	....	6.0	....	6.0	5.7	5.8
K_2SO_4	....	....	6.1	6.2	5.9	5.8
Displaced soil solution	....	6.2	5.6	5.7	5.7	5.8
Check	7.1	6.5	5.9	5.7	5.9	6.1
S & CaO	7.0	6.6	5.6	5.7	5.8	6.0
S	6.8	6.6	5.4	5.6	5.8	6.3
Gypsum	7.0	6.9	5.7	5.5	5.9	6.2
K_2SO_4	7.0	6.9	5.7	5.9	6.0	6.2
Check	6.9	6.6	6.2	5.6	6.0	6.0
$(NH_4)_2SO_4$	7.0	6.5	6.0	5.6	6.0	6.1
$MgSO_4$	7.1	7.0	5.7	5.8	6.2	6.2
CaO	7.1	7.2	6.1	6.0	6.2	6.2
S & CaO	7.0	6.9	5.9	5.8	6.0	6.3
S	7.0	6.7	5.8	4.9	6.0	6.2
Gypsum	....	6.0	....	6.2	5.7	6.2
K_2SO_4	....	....	6.0	5.6	5.7	5.8
Displaced soil solution	....	6.3	6.0	5.9	5.9	6.1

total growth was secured for legumes at a pH of 5.2 to 6.5, the range being wider for alfalfa than for alfalfa bacteria. Water culture studies with rhododendrons indicate that they grow best at a pH of about 5.0 and that even 2 p.p.m. of aluminum is toxic (plate 2, fig. 2).

Mint tends to control a reaction promptly or does not survive if prevented from bringing about and maintaining a favorable reaction (plate 2, fig. 1).

The hydrogen-ion concentration of field soils where the crops were flourishing was determined in several cases with most of the plants studied. The plants studied were found to flourish in a slightly acid medium.

The roots in contact with the soil solution may cause the reaction to change to a slight acidity because of the production of CO_2 and then H_2CO_3.

TABLE 3

Yield of seedlings grown on treated Delhi soil in pots, February 3 to May 27

TREATMENT	YIELD JAR 1	YIELD JAR 2	YIELD JAR 3	TOTAL YIELD	MEAN YIELD OF EACH JAR	WATER REQUIREMENT	REACTION
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*pH*
Displaced soil..........	4.65	....	4.96	9.61	4.81	1,332	5.8
K_2SO_4................	5.41	....	5.90	11.31	5.26	1,216	5.8
$CaSO_4$................	5.44	5.01	5.47	15.82	5.16	1,238	6.2
S—100 pounds Acre....	6.68	6.54	6.51	19.73	6.58	971	6.2
S + CaO.............	6.31	7.25	7.14	20.70	6.90	928	6.3
CaO..................	6.21	7.31	7.28	20.80	6.94	922	6.4
$(NH_4)_2SO_4$............	5.66	6.08	6.95	18.69	6.12	1,045	6.0
$MgSO_4$...............	6.02	6.90	6.84	19.76	6.59	971	6.1
Check untreated.......	4.90	5.86	6.04	16.80	5.30	1,208	6.0

Where sulfur, sulfate, and other treatments were applied which tended to develop an acid reaction, this was associated with an increase in growth. The alfalfa plants tended to bring the reaction to about pH 6.0.

Optimum reaction for these plants when grown in the field or in soil pots seems to correspond to the optimum reaction for these same plants when grown in culture solutions. Full advantage should be taken of the soil reaction range in which resistant crops grow well.

REFERENCE

(1) BRYAN, O. C. 1923 Effect of reaction on growth, nodule formation and calcium content of alfalfa, alsike clover and red clover. *Soil Sci.* 15:23–30.

PLATE 1

ALFALFA, VETCH, AND ALSIKE GROWN IN CULTURE SOLUTIONS OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

FIG. 1. Alfalfa. FIG. 2. Alsike. FIG. 3. Vetch.

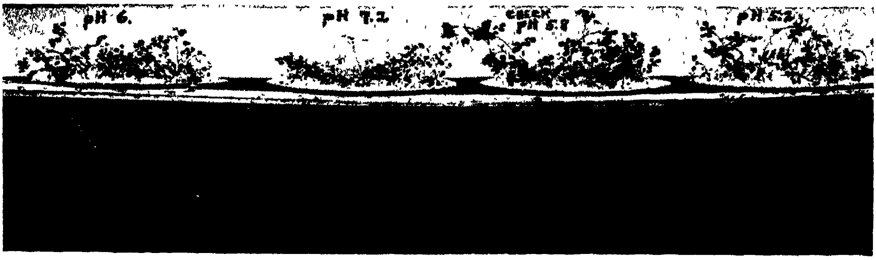

Fig. 1

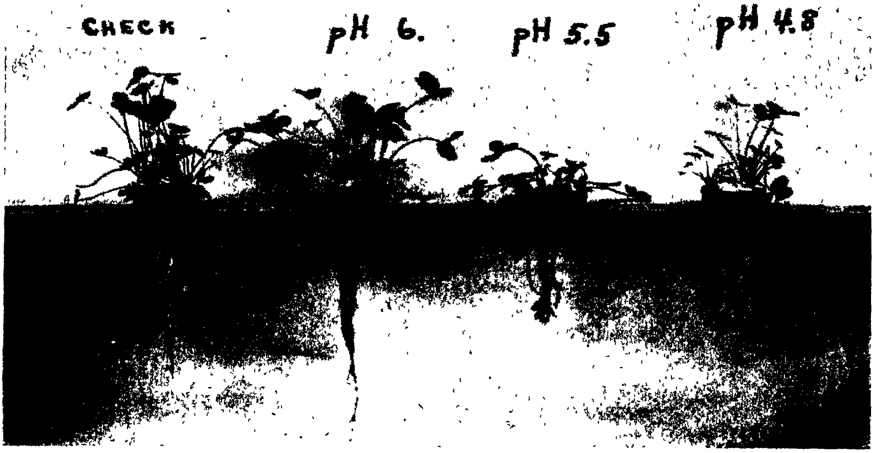

Fig. 2

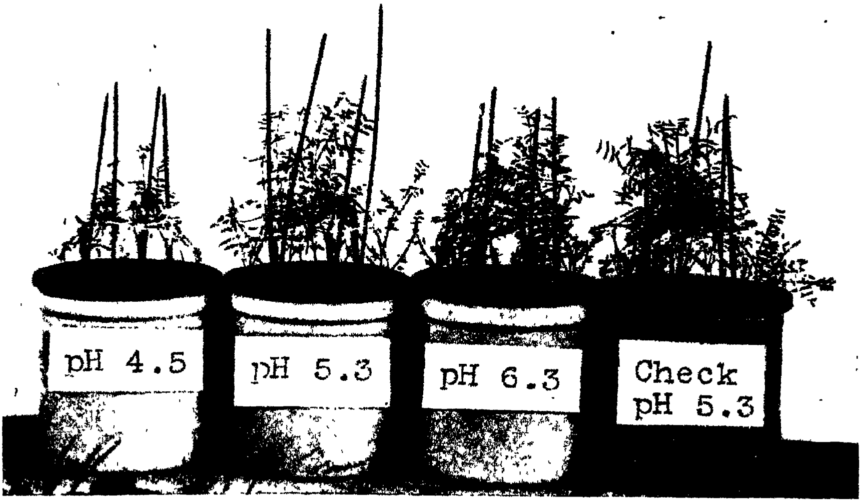

Fig. 3

PLATE 2

SPEARMINT AND RHODODENDRON GROWN IN CULTURE SOLUTIONS OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

FIG. 1. Spearmint.
FIG. 2. Rhododendron.

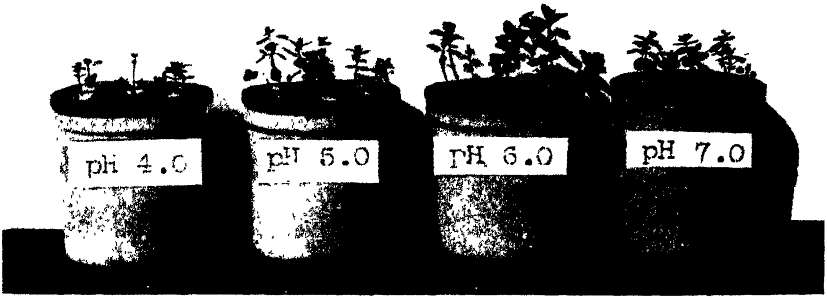

FIG. 1

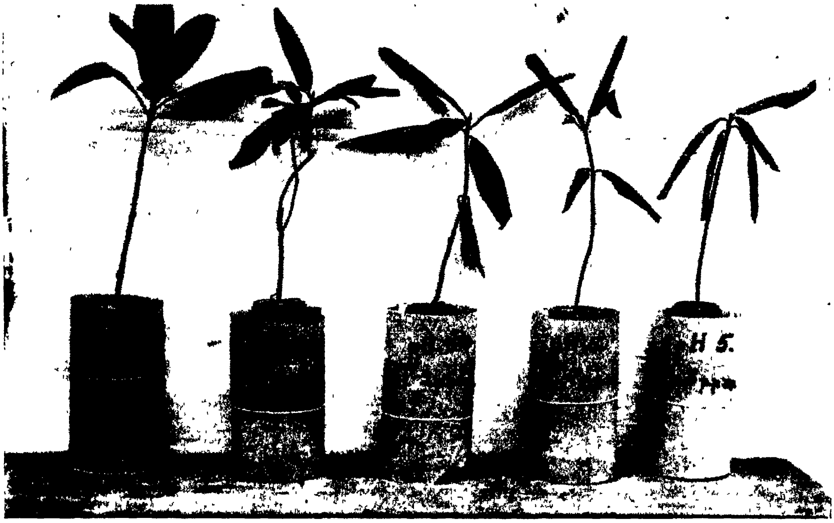

FIG. 2

HILL FERTILIZATION STUDIES ON THE UTILIZATION OF THE PHOSPHORUS IN ACID PHOSPHATE BY CORN

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At present there seems to be a decided difference of opinion among investigators in regard to the best method of applying fertilizers. Some experiments (3, 5) have indicated that the proper application of fertilizer "in the hill" with corn, and in the row with other crops, brought about greater increases in yields, than were obtained when the same amounts of fertilizer were applied broadcast. Other experiments (2) conducted under different conditions have indicated that broadcasting is to be preferred to hill or row applications.

Although the increase in crop yield must determine the best method of application, it is quite possible that if more information were available on the residual effect of fertilizers, this factor might also help to determine which method should be used. In the case of broadcast applications, especially of acid phosphate, if the first crop does not utilize all of the plant-food added, the remainder will be distributed so that it can be used by succeeding crops. In hill or row fertilization, if all of the plant-food applied is not used by the crop being grown, it is left in a small area of soil and only a few of the roots of succeeding crops can come in contact with it. If a crop can utilize practically all of the fertilizer in the hill or row, however, the residual effect would not be a factor in determining how fertilizers should be applied.

Few data (1) have been published on the utilization of phosphorus in the hill or row. Hence it seemed desirable to study this problem further in order to determine, if possible, how much of the phosphorus added was used by the growing crop.

EXPERIMENTAL

In this investigation a study was made on the utilization of the phosphorus by corn when fertilized in the hill with 16 per cent acid phosphate and a 2-12-2 fertilizer made up of ammonium sulfate, 16 per cent acid phosphate, and potassium chloride. The mixed fertilizer was included in order to determine whether nitrogen and potassium used with the acid phosphate would increase the utilization of the phosphorus, through stimulating the development of fine roots in the young corn plant, particularly in the fertilized zone.

The acid phosphate was ground to pass a 40-mesh sieve and was applied at the rate of 100 pounds to the acre, or 12½ gm. to the hill. The 2-12-2 fertilizer

TABLE 1

The total nitrogen and total phosphorus content, and the acidity of the soils used in determining the utilization of phosphorus by corn in hill fertilization studies

NUMBER	SOIL TYPE	LOCATION	FIELD	TOTAL N PER 2,000,000 POUNDS OF SOIL	TOTAL PHOSPHORUS PER 2,000,000 POUNDS OF SOIL	ACIDITY BY TRUOG TEST
1	Carrington loam	Story County	Agronomy Farm	2,760	610	Slight
2	Carrington loam	Bremer County	Waverly No. 2	3,560	970	Strong
3	Clinton silt loam	Muscatine County	Sherfey	3,380	720	Slight+
4	Marion silt loam	Lee County	West Point No. 2	1,980	550	Medium+
5	Marshall silt loam	Pottawattamie County	Avoca	3,980	1,120	Slight+
6	Marshall silt loam	Woodbury County	Lawton	3,430	1,110	Slight+
7	Peat	Buena Vista County	Rembrandt	16,070*	780*	Slight
8	Tama silt loam	Winneshiek County	Ossian	4,420	1,190	Medium

* Pounds to every 1,000,000 pounds of soil.

was applied at the rate of 133.3 pounds to the acre, equivalent to 100 pounds of 16 per cent acid phosphate. The fertilizers were applied by hand and thoroughly mixed with a layer of soil 3 inches wide, 12 inches long, and in most cases about three-fourths of an inch thick. A 4-mesh, black wire screen was placed below and above the fertilized zone to facilitate accurate sampling of soil of the fertilized area after the corn had grown to maturity. The corn was planted in the middle of the fertilized area at a depth of about two and one-half inches. No injury to germination occurred, and in most cases there were three stalks of corn in each hill.

The tests were carried out on six different soil types located in eight different counties in Iowa. All of the fertilizer treatments were made and the corn was planted about May 5. After the corn was matured, the plants were removed and soil samples were taken from the fertilized areas between the wire screens, and from areas one inch deep directly beneath each lower screen. A composite sample of unfertilized soil was also obtained from the area between the fertilized hills. All samples were analyzed for total phosphorus content by the magnesium nitrate method. The unfertilized samples were also analyzed for total nitrogen by the Kjeldahl method and for acidity by the Truog test (4).

The results of the analyses of the unfertilized soils are given in table 1. The data obtained from the fertilized samples are given in table 2. The amount of residual phosphorus was calculated by determining the amount of phosphorus remaining after crop maturity in each sample of fertilized soil, and then subtracting the amount of phosphorus which was present in a like amount of unfertilized soil. Ninety milligrams of phosphorus was added to each hill of corn. The percentage of phosphorus which was not used by the corn crop was obtained by dividing the residual phosphorus by 0.9.

The data given in table 2, indicate that when soils are high in total phosphorus, the corn plant uses less phosphorus from the fertilizer applied in the hill than when soils are low in total phosphorus. Soil acidity did not seem to affect the utilization of phosphorus in this investigation. The Marshall silt loam at Lawton received very little rainfall during the spring and summer, which may account for the large amount of residual phosphorus in that soil. Samples of soil were not obtained from the area beneath the fertilized zone in the Tama silt loam, Ossian Field, where the soil was fairly high in total phosphorus. A large amount of the phosphorus added to this soil was utilized by the corn plant. The better utilization of the phosphorus was probably due to the mixing of the fertilizer with a layer of soil about two inches instead of three-quarters of an inch thick, as was done in the other tests. A larger number of plant roots thus came in contact with the thicker layers of fertilizer. It is evident from this investigation, which confirms the results previously secured (1), that the corn plant has not been able to utilize, even under the most favorable conditions, all of the phosphorus in an application of 100 pounds of 16 per cent acid phosphate applied in the hill in the same plane with the seed. It was also found in connection with this study that the amount of

TABLE 2

Hill fertilization data on the residual phosphorus which remained in the soil after a crop of corn had grown to maturity

FERTILIZER APPLIED	RESIDUAL PHOSPHORUS			PHOSPHORUS APPLIED
	In fertilized zone	Beneath fertilized zone	Total	
	mgm.	*mgm.*	*mgm.*	*per cent*
		Carrington loam, Agronomy Farm		
0-16-0	22.5	2.5	25.0	27.8
	23.1	3.8	26.9	29.9
	18.6	5.8	24.4	27.1
	18.3	9.4	27.7	30.8
Average.......	20.6	5.4	26.0	28.4
2-12-2	11.6	3.5	15.1	16.8
	16.7	1.6	18.3	20.3
	23.8	2.8	26.4	29.3
	28.7	0.5	29.2	32.4
Average.......	20.2	2.1	22.3	24.7
		Carrington loam, Waverly Field No. 2		
0-16-0	20.5	6.0	26.5	29.4
	23.3	7.1	30.4	33.8
	23.5	6.7	30.2	33.6
	16.4	4.7	21.1	23.4
Average.......	20.9	6.1	27.0	30.0
2-12-2	35.6	6.5	42.1	46.8
	20.7	1.9	22.6	25.1
	32.4	8.6	41.0	45.6
	29.8	7.7	37.5	41.7
Average.......	29.6	6.2	35.8	39.8
		Clinton silt loam, Sherfey Field		
0-16-0	18.8	11.3	30.1	33.4
	11.3	10.4	21.7	24.1
	8.3	7.0	15.3	17.0
	12.4	6.3	18.7	20.8
Average.......	12.7	8.7	21.4	23.8
2-12-2	10.7	8.4	19.1	21.2
	12.1	5.8	17.9	19.9
	10.1	12.3	22.4	24.9
	12.2	0.0	12.2	13.5
Average.......	11.3	6.6	17.9	19.9

TABLE 2—*Continued*

FERTILIZER APPLIED	RESIDUAL PHOSPHORUS			PHOSPHORUS APPLIED
	In fertilized zone	Beneath fertilized zone	Total	
	mgm.	*mgm.*	*mgm.*	*per cent*
		Marion silt loam, West Point Field No. 2		
0-16-0	13.1 10.8 15.7 9.2	12.5 9.5 3.7 18.3	25.6 20.3 19.4 27.5	28.4 22.6 21.5 24.7
Average.......	12.2	11.0	23.2	24.3
2-12-2	6.8 11.4 18.4 18.1	2.3 5.8 7.7 5.4	9.1 17.2 26.1 23.5	10.1 19.1 29.0 26.1
Average.......	13.7	5.3	19.0	21.1
		Marshall silt loam, Avoca Field		
0-16-0	25.5 32.3 24.2 23.1	2.1 5.5 5.1 8.0	27.6 37.8 29.3 31.1	30.7 42.0 32.6 34.6
Average.......	26.3	5.2	31.5	35.0
2-12-2	23.4 27.6 42.0 27.4	4.8 5.7 5.7 3.9	28.2 33.3 47.7 31.3	31.3 37.0 53.0 34.8
Average.......	30.1	5.0	35.1	39.0
		Marshall silt loam, Lawton Field		
0-16-0	33.1 36.3 36.1 30.6	2.9 6.8 0.7 3.6	36.0 43.1 36.8 34.2	40.0 47.9 40.9 38.0
Average.......	34.0	3.5	39.5	41.7
2-12-2	43.9 34.1 44.5 41.4	0.0 0.9 0.7 0.0	43.9 35.0 45.2 41.4	48.8 38.9 50.2 46.0
Average.......	41.0	0.4	41.4	46.0

TABLE 2—*Concluded*

FERTILIZER APPLIED	RESIDUAL PHOSPHORUS			PHOSPHORUS APPLIED
	In fertilized zone	Beneath fertilized zone	Total	
	mgm.	*mgm.*	*mgm.*	*per cent*
Peat, Rembrandt Field				
0-16-0	10.6	2.4	13.0	14.4
	2.2	2.2	4.4	4.9
	18.5	1.5	20.0	22.2
	16.4	3.6	20.0	22.2
Average.......	11.9	2.4	14.3	15.9
2-12-2	13.3	0.9	14.2	15.8
	17.8	3.9	21.7	24.1
	8.8	0.0	8.8	9.8
	4.9	0.3	5.2	5.7
Average.......	11.2	1.3	12.5	13.8
Tama silt loam, Ossian Field				
0-16-0	23.9	....	23.9	26.5
	13.1	....	13.1	14.5
	17.1	....	17.1	19.0
	15.8	....	15.8	17.6
Average.......	17.5	....	17.5	19.4
2-12-2	21.7	....	21.7	24.1
	25.3	....	25.3	28.3
	19.3	....	19.3	21.4
	17.4	....	17.4	19.3
Average.......	20.9	....	20.9	23.2

residual phosphorus was greater when fertilizer attachments were used than when the fertilizer was applied by hand. This probably is because most fertilizer attachments do not mix the fertilizer thoroughly with the soil. The data along this line are not given in this paper. Another fact which does not appear in the data but which was observed during this study was that a greater amount of phosphorus was used when the fertilizer was located 2 inches instead of 6 inches from the center of the hill. This is evidently because of the difference in the concentration of roots in these areas, since there were more fine roots 2 inches from the center of the hill than at a distance of 6 inches. This would seem to indicate that in hill fertilization, phosphorus fertilizers should be applied in the zone of maximum root development and should be thoroughly mixed with the soil in order to obtain the best utilization of the plant-food in the fertilizer.

SUMMARY

A study was made of the utilization of the phosphorus in acid phosphate by corn plants which were fertilized in the hill with 16 per cent acid phosphate and with a 2-12-2 fertilizer.

It was found that about three-fourths of the phosphorus applied was taken up by the corn plant when the rate of fertilization was equivalent to 100 pounds of 16 per cent acid phosphate to the acre. The amount of phosphorus absorbed varied with the different soils and was greater than 75 per cent in case of soils low in phosphorus, and less than 75 per cent in soils high in phosphorus. The lack of available nitrogen probably reduced the utilization of phosphorus in two cases.

The maximum utilization of phosphorus was found to occur in the zone of greatest root development, which in the cases studied was less than six inches from the center of the corn hill.

The addition of ammonium sulfate and potassium chloride to acid phosphate to make a 2-12-2 fertilizer did not increase the total absorption of phosphorus from acid phosphate when applied in the hill to corn. It may, however, have had some effect on the rate of absorption, which was not determined in this investigation.

REFERENCES

(1) Harper, H. J. 1925 A study of the secondary effects of hill fertilization. Ia. Agr. Exp. Sta. Res. Bul. 87.

(2) Miller, M. F. 1923 Fertilizing the corn crop. *Amer. Fert.* 59 (3): 32–33.

(3) Mooers, C. A. 1903 Fertilizer experiments. Bul. Tenn. Agr. Exp. Sta., v. 16: 16–18.

(4) Truog, E. 1920 Testing soils for acidity. Wis. Agr. Exp. Sta. Bul. 312.

(5) Truog, E., et al. 1925 Fertilizer experiments. Wis. Agr. Exp. Sta. Res. Bul. 65.

THE ASSIMILATION OF PHOSPHORUS FROM PHYTIN BY RED CLOVER[1]

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In an earlier paper (3), oats were shown to assimilate phosphorus from phytin. This form of organic phosphorus was more readily assimilated than that of certain inorganic phosphates. The straw of oats was found to contain as high as 1.94 per cent of phosphorus with phytin, which in every treatment with the crude phytin, as well as with the pure phytin, exceeded that found with even the largest application of rock phosphate.

The oat grain showed a generally higher phosphorus content with the largest applications of phytin, possibly indicating a very great tolerance in the straw for the phosphorus in the phytin treatments. The phosphorus content of oat straw is usually reported as 2 pounds a ton, although some of these results showed as high as 38 pounds a ton, with the lowest of the phytin treatments showing 3.2 pounds a ton.

Clover follows oats in many crop rotations in which corn precedes the oats. The clovers have been shown to be capable of using insoluble sources of phosphorus and may obtain considerable organic phosphorus from the stubble and straw of the oats and the stalks of the corn.

In order to test the ability of clover to assimilate phosphorus from phytin the experiments reported in this paper were conducted.

Tsuda (2) has reported the phosphorus distribution in red clover hay before flowering as follows:

	In 100 parts dry matter	
Total P_2O_5		0.554
As phytin	0.300	
As other organic	0.084	
As lecithin	0.050	
As nuclein	0.050	
As inorganic	0.070	

About one half of the total phosphorus in the red clover was reported as phytin. These data are introduced to show the importance of that source of phosphorus to crops that must depend upon clover for their phosphorus.

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[1] Work conducted at the University of Illinois. Submitted by the senior author to the Graduate School in partial fulfillment of the requirements for the degree of Master of Science.

EXPERIMENTAL

The pot-culture methods were outlined in the previous paper. These clover series were conducted in a similar manner to the oat series reported therein.

Clover Series 1–28—Set up immediately before planting

Phosphorus applications

1–2—Sand alone, no treatment
3–4—These and all succeeding pots received the following treatment:
    28 gm. precipitated calcium carbonate
    Soluble potassium, magnesium, and nitrogen
    Liberal application of soil infusion
Rock Phosphate Series
    5– 6— 220 mgm. phosphorus
    7– 8— 440 mgm. phosphorus
    9–10— 880 mgm. phosphorus
    11–12—2,640 mgm. phosphorus
Tricalcium Phosphate Series
    13–14— 36 mgm. phosphorus
    15–16— 73 mgm. phosphorus
    17–18—146 mgm. phosphorus
    19–20—440 mgm. phosphorus
Crude Phytin Series
    21–22— 18 mgm. phosphorus
    23–24— 36 mgm. phosphorus
    25–26— 73 mgm. phosphorus
    27–28—220 mgm. phosphorus

Series 29–56

The treatments on series 29–56 are obtained by subtracting from the total number of milligrams of phosphorus originally placed in each pot, the number of milligrams of phosphorus removed in the grain and straw of the oats crop. Aside from leaching, which perhaps can be disregarded, there was no possible loss.

These series were planted to red clover on January 26. In the seed planted, about 0.2 mgm. of phosphorus was added to each pot. The clover was allowed to grow until it was evident that it would live when it was thinned to 12 plants to each pot. The red spider and white fly were held in check by daily spraying with cold distilled water. Some injuries which were thought to be due to ammonium nitrate were noticed, but were not permanent and were not seen on series 29–56.

On April 26 the first cutting was made. It will be noted, however, that because the plants were too small, the following pots were not harvested:

Checks	1–4
Tricalcium phosphate	13–20
Checks	29–32
Rock phosphate	33–38 and 40

Table 1 gives yields of the first cutting together with the percentage and the total number of milligrams of phosphorus in the crop. The figures are averages of the two duplicate pots.

Plates 1 and 2 show the clover at the time of the first cutting.

The increasing amounts of phosphorus in all forms used resulted in increased yields. The percentages of phosphorus in the first cutting increased in general with increasing amounts of phosphorus applied. In series 41–48 and 49–56 very high phosphorus contents were found. Both these series followed oats, and the phosphorus given as treatment is the amount remaining after that removed by the oats is subtracted. The clover did not take up as much phosphorus in any case as did the oats, the highest phosphorus content being 0.895 per cent with the clover. Clover hay was reported by Hopkins as containing 5 pounds of phosphorus to the ton. The phytin treatments

TABLE 1

Yields and analyses of the first cutting of clover

POT NUMBER	TREATMENT, P PER POT	YIELD	PHOSPHORUS	TOTAL PHOSPHORUS IN CROP
	mgm.	*gm.*	*per cent*	*mgm.*
		Rock Phosphate Series		
5–6	220	0.48	0.154	0.74
7–8	440	0.82	0.179	1.47
9–10	880	1.57	0.175	2.75
11–12	2,640	3.91	0.212	8.29
		Crude Phytin Series		
21–22	18	0.74	0.160	1.18
23–24	36	1.65	0.253	4.17
25–26	73	3.26	0.330	10.75
27–28	220	6.40	0.335	21.44
		Rock Phosphate Series (after oats)		
39	2,520	0.79	0.266	2.10
		Pure Phytin Series (after oats)		
41–42	132	0.65	0.522	3.39
43–44	297	1.38	0.555	7.66
45–46	650	2.18	0.691	15.06
47–48	2,341	2.08	0.853	17.74
		Crude Phytin Series (after oats)		
49–50	119	0.38	0.615	2.34
51–52	239	0.44	0.895	3.94
53–54	516	1.76	0.768	13.51
55–56	2,040	5.00	0.678	33.90

after oats in this work contained from 10.4 to 17.9 pounds to the ton—from two to over three times as much as commonly found. The intake of phosphorus by red clover from phytin was thus very large.

On May 15 a second cutting was made and the roots were removed and washed as free from sand as possible. It was impossible to remove all of the sand but what remained did not interfere seriously with the reliability of the weights. In the tables where total crop weights are reported they represent the entire plant. Table 2 gives the yields on clover series 1–28. A very small amount of phosphorus in phytin was equivalent in yield to a large amount of either tricalcium phosphate or rock phosphate. Table 3 gives the yields on the series where the clover followed a crop of oats (29–56).

TABLE 2

Yields of clover on series 1-28

POT NUMBER	TREATMENT, P PER POT	TOPS		ROOTS	TOTAL
		First cutting	Second cutting		
	mgm.	*gm.*	*gm.*	*gm.*	*gm.*
Checks					
1–2	None	.....	.....	.....	0.39
3–4	All but P	.....	.....	.....	0.28
Rock Phosphate Series					
5–6	220	0.48	0.57	1.17	.....
7–8	440	0.82	1.02	1.60	.....
9–10	880	1.57	1.86	3.00	.....
11–12	2,640	3.91	3.70	5.68	.....
Tricalcium Phosphate Series					
13–14	36	.....	.....	.....	0.50
15–16	73	.....	.....	.....	0.40
17–18	146	.....	.....	.....	0.56
19–20	440	.....	.....	.....	0.90
Crude Phytin Series					
21–22	18	0.74	0.78	1.63	.....
23–24	36	1.65	1.95	2.79	.....
25–26	73	3.26	3.20	3.96	.....
27–28	220	6.40	2.96	5.70	....

In series 33–40 with the second crop on the rock phosphate or where the clover followed oats, it will be seen that the low yields produced are in a way identical with those on the tricalcium phosphate series 13–20. An exception exists, however, with pots 39 and 40, but this may be explained by the fact that the roots of the oats were not removed. In these two pots, judging from the amount of oat straw produced, considerable quantities of roots must have been left, which, on decomposition, liberated organic phosphorus which was utilized by the clover. The percentage phosphorus content of the clover on these pots would also indicate organic origin. It will be noted that most of the growth was during the last month, which indicates that growth was delayed until the phosphorus was liberated by decomposition.

TABLE 3

Yields of clover on series 29-56 (after oats)

POT NUMBER	TREATMENT, P PER POT	TOPS		ROOTS	TOTAL
		First cutting	Second cutting		
	mgm.	*gm.*	*gm.*	*gm.*	*gm.*
		Checks			
29–30	None	.....	.....	.....	.....
31–32	All but P	.....	.....	.....	0.51
		Rock Phosphate Series			
33–34	210	.....	.....	.....	0.52
35–36	421	.....	.....	.....	0.75
37–38	840	.....	.....	.....	1.06
39	2,520	0.79	2.68	4.34	.....
40	2,520	.....	0.92	2.75	.....
		Pure Phytin Series			
41–42	132	0.65	1.92	2.75	.....
43–44	297	1.38	2.51	3.45	.....
45–46	650	2.18	3.85	4.36	.....
47–48	2,341	2.08	4.07	5.78	.....
		Crude Phytin Series			
49–50	119	0.38	1.14	1.92	.....
51–52	239	0.44	Removed for analysis		.....
53–54	516	1.76	3.09	4.73	.....
55–56	2,040	5.00	4.22	5.89	.....

ORGANIC PHOSPHORUS ANALYSIS

In the crude phytin series on which clover followed oats, it was noticed that the clover crop was poorest on the lower treatments where the best crop of oats was produced, although a sufficient amount of phosphorus was present to grow a maximum crop. Originally it was thought that all of the phosphorus in crude phytin was organic but this phenomenon raised the question whether part of the phosphorus in crude phytin might not be inorganic. Collison (1) had separated organic and inorganic phosphates by the use of acid alcohol. His method was tried in this case. He used 94–96 per cent alcohol containing 0.2 per cent hydrochloric acid. The finely ground substance is shaken up with this acid alcohol solution for several hours or until all of the inorganic phosphorus goes into solution, the difference between the total phosphorus and that in solution being organic.

Where mineral phosphates were to be separated, it was found that 0.2 per cent hydrochloric acid was not sufficiently strong to dissolve all of the inorganic phosphorus. The acid was then increased to 1 per cent, thus making a final solution of about 94 per cent alcohol containing 1 per cent hydrochloric acid.

A weighed amount of the substance was placed in a shaker bottle with 200 cc. of the 1 per cent acid alcohol, shaken at intervals and allowed to stand until the reaction had reached an equilibrium, which on the mineral phosphates was as long as ten days or two weeks. Trial separations were made on phytin and pure tricalcium phosphate and the method was checked up in this way.

TABLE 4

Trial separation of organic and inorganic phosphorus

SUBSTANCE	WEIGHT OF SAMPLE	INORGANIC P	ORGANIC P
	gm.	*mgm.*	*mgm.*
Separately:			
$Ca_3(PO_4)_2$	0.05	7.31	....
Phytin	0.05	0.84	5.64
Together:			
$Ca_3(PO_4)_2$	0.05	8.20	5.59
Phytin	0.05		

The results in table 4 are averages of four determinations and check within the limit of experimental error. They indicate a complete separation in 1 per cent acid alcohol.

In view of the fact that the trial separation was so complete and accurate, a separation was tried on pots 51 and 52, on which only a small amount of clover was produced, but on which had grown the maximum oats crop of this series. In the beginning, 351 mgm. of phosphorus as crude phytin was added to each pot. According to the above analysis this contained only 305 mgm. of organic phosphorus. The total oats crop removed in the grain and straw was 112 mgm. of phosphorus. The clover crop removed in all 11 mgm. of phosphorus, making a total 123 mgm. of phosphorus removed by the crops, and leaving 182 mgm. of presumably organic phosphorus together with the 46 mgm. of inorganic phosphorus applied in the phytin at the beginning. As an average of four determinations on the sand from pots 51 and 52, only 31 mgm. of organic phosphorus to the pot could be found. This shows that at least 151 mgm. of organic phosphorus had gone over to the inorganic state, and in the presence of lime presumably to tricalcium phosphate. The total weight of phosphorus assimilated in the first cutting from pots 51 and 52 checks very closely with that assimilated in pots 23 and 24 into each of which was placed 36 mgm. of phosphorus in the form of phytin and carrying 31.3 mgm. of organic phosphorus.

It was also noticed early that the clover grown on the rock phosphate series which had previously grown oats, did not make a growth equal to that on rock phosphate which had not previously grown oats (*see* tables 2 and 3). The difficulty seemed to be similar to that affecting the crude phytin clover. Since the rock phosphate and phytin had been treated exactly alike and had both shown the same phenomenon, namely, that of reduced yields, it was considered worth while to attempt to determine whether the rock phosphate contained any organic phosphorus. Samples of rock phosphate were run for organic phosphorus with the results given in table 5. This shows that 5.1 per cent of the phosphorus in rock phosphate is organic, or 0.62 per cent of the mineral is organic phosphorus. There is the possibility, however, that this figure is too high, but coupled with the fact that rock phosphate may contain over 6 per cent of organic matter, would indicate perhaps that it does contain some organic phosphorus. In this connection it might be well to note that rock phosphate contains about 0.5 mgm. of organic nitrogen in each gram. These points together with the fact that the clover following oats on the rock phos-

TABLE 5

Organic phosphorus in rock phosphate

SUBSTANCE	WEIGHT OF SAMPLE	PHOSPHORUS	
	gm.	*mgm.*	*per cent*
Total phosphorus	0.05	6.06	12.12
Acid alcohol soluble	0.05	5.75	11.50

TABLE 6

Comparative yields and treatments on rock phosphate and crude phytin

POT NUMBER	TREATMENT	ORGANIC PHOSPHORUS	YIELD	PHOSPHORUS* FIRST CUTTING
		mgm.	*gm.*	*mgm.*
7–8	Rock phosphate	26.4	1.84	1.47
21–22	Crude phytin	15.6	1.52	1.18
9–10	Rock phosphate	52.8	3.43	2.75
23–24	Crude phytin	31.3	3.60	4.17
11–12	Rock phosphate	158.4	7.60	8.29
25–26	Crude phytin	63.5	6.48	10.75

* From table 1.

phate was inferior, all indicate some form of readily available phosphorus in the rock, which tends in the presence of lime to go to the tricalcium or other unavailable form. The nitrogen in rock phosphate is low in comparison with the organic matter and the organic phosphorus, and this would tend to indicate non-nitrogenous phosphates such as phytin.

By taking the sum of the first and second cuttings of clover on similarly producing pots in the rock phosphate and phytin series, and computing from the treatment the amount of organic phosphorus present, it will be seen that there is sufficient organic phosphorus in the rock to produce these yields. A marked similarity exists in the amounts of organic phosphorus present in the two treatments. Table 6 shows the number of milligrams of organic phosphorus (by calculation) present in each pot, the total yield from the two cuttings, and the number of milligrams assimilated in the first cutting. All of these data tend to show that the rock phosphate used in this work contained enough organic phosphorus to grow the crops produced, without using any of the inorganic form.

SUMMARY

1. Red clover is able to utilize phosphorus supplied in phytin.
2. Organic phosphorus in rock phosphate appeared to be responsible for increased growth with the first crop of red clover.
3. Phosphorus in the roots of oats appeared to be an important source of that element for the succeeding crop.
4. The presence of lime brings about a reversion of the soluble organic phosphorus to a less available inorganic form. This appears to take place even with the rock phosphate.

REFERENCES

(1) Collison, R. C. 1912 Inorganic phosphorus in plant substances. An improved method of estimation. *Jour. Biol. Chem.* 12: 65.

(2) Tsuda, S. 1909–13 On the different forms of phosphoric acid in organic manures. *Jour. Col. Agr., Imp. Univ. Tokyo* 1: 167.

(3) Whiting, A. L., and Heck, A. F. 1926 The assimilation of phosphorus from phytin by oats. *Soil Sci.* 22: 477–494.

PLATES

PLATE 1

Clover at the Time of First Cutting

Fig. 1. Pot 4—All plant-food except P
22— 18 mgm. P in crude phytin to the pot
24— 36 mgm. P in crude phytin to the pot
26— 73 mgm. P in crude phytin to the pot
28— 220 mgm. P in crude phytin to the pot

2 —No treatment
14— 36 mgm. P in tricalcium phosphate to the pot
16— 73 mgm. P in tricalcium phosphate to the pot
18— 146 mgm. P in tricalcium phosphate to the pot
20— 440 mgm. P in tricalcium phosphate to the pot

Fig. 2. Pot 32—All plant-food except P
50— 119 mgm. P in crude phytin to the pot
52— 239 mgm. P in crude phytin to the pot
54— 516 mgm. P in crude phytin to the pot
56—2,040 mgm. P in crude phytin to the pot

30—No treatment
42— 132 mgm. P in pure phytin to the pot
44— 297 mgm. P in pure phytin to the pot
46— 650 mgm. P in pure phytin to the pot
48—2,341 mgm. P in pure phytin to the pot

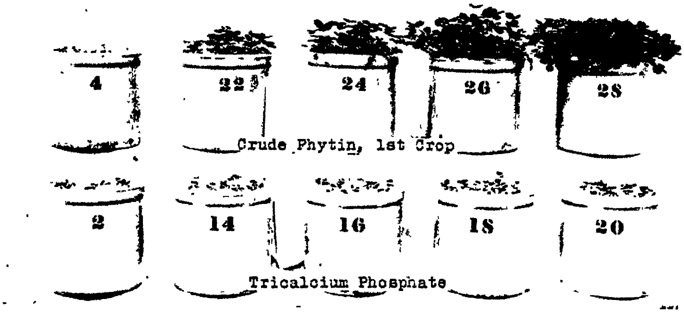
4
22
24
26
28
Crude Phytin, 1st Crop
2
14
16
18
20
Tricalcium Phosphate

FIG. 1

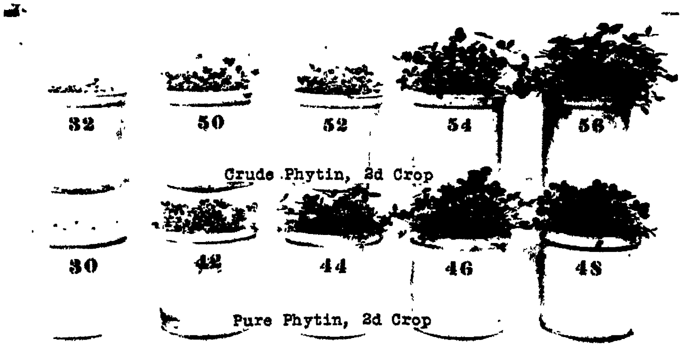
32
50
52
54
56
Crude Phytin, 2d Crop
30
42
44
46
48
Pure Phytin, 2d Crop

FIG. 2

PLATE 2

Clover at the Time of First Cutting

Fig. 1. Pot 4—All plant-food except P
32—All plant-food except P
20— 440 mgm. P in tricalcium phosphate to the pot
36— 421 mgm. P in rock phosphate to the pot
8— 440 mgm. P in tricalcium phosphate to the pot
28— 220 mgm. P in crude phytin to the pot

22— 18 mgm. P in crude phytin to the pot
8— 440 mgm. P in crude phytin to the pot
24— 36 mgm. P in crude phytin to the pot
10— 880 mgm. P in rock phosphate to the pot
26— 73 mgm. P in crude phytin to the pot
12—2,640 mgm. P in rock phosphate to the pot

Fig. 2. Pot 2—No treatment
4—All plant-food except P
6— 220 mgm. P in rock phosphate to the pot
8— 440 mgm. P in rock phosphate to the pot
10— 880 mgm. P in rock phosphate to the pot
12—2,640 mgm. P in rock phosphate to the pot

30—No treatment
32—All plant-food except P
34— 210 mgm. P in rock phosphate to the pot
36— 421 mgm. P in rock phosphate to the pot
38— 840 mgm. P in rock phosphate to the pot
40—2,520 mgm. P in rock phosphate to the pot

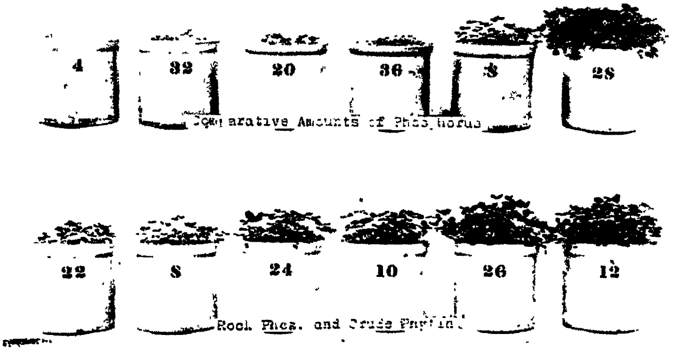
4
32
20
36
8
28
Comparative Amounts of Phosphorus
22
8
24
10
26
12
Rock Phos. and Crude Phytin

FIG. 1

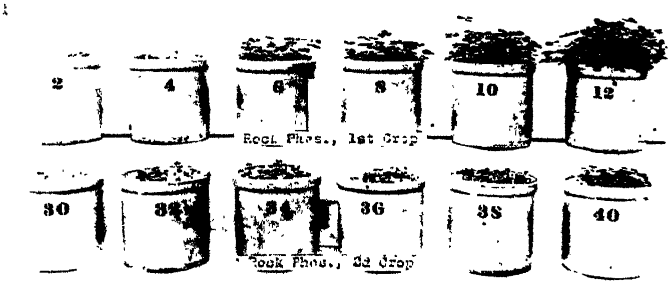
2
4
6
8
10
12
Rock Phos., 1st Crop
30
32
34
36
38
40
Rock Phos., 2d Crop

FIG. 2

THE RELATIVE RATES OF NITRIFICATION OF DIFFERENT PARTS OF SWEET CLOVER PLANTS[1]

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In the progress of agriculture there is noted a tendency toward more complete and more intelligent utilization of crop residues and green manures both from the soil standpoint and from that of the farm as a unit.

This tendency gives rise to many new questions dealing with the relative rates of decomposition of the different crops and the different parts of the same crop. These questions are concerned with dry and green, young and old, cured and uncured (6) legume and non-legume plants and parts of plants under the influence of the cropping and soil systems being practiced.

There is unquestionably much to be gained by a more complete knowledge of the rate and kind of decomposition or fermentation of the various crop residues and green manures and the parts thereof.

This paper deals with only one phase of this whole question of decomposition and for one crop only, although it touches on perhaps the most important element from the soil standpoint.

The work reported consists of a study of the relative rates of nitrification of the whole plant and the parts of the biennial white sweet clover of the first season's growth.

Joshi (2) studied the legumes, sann-hemp, dhaincha, cowpeas, tamarind, guvar, and gokarn and found the rate of nitrification greatest for the leaves. The roots and stems were very slow to be nitrified. These are all annual legumes except tamarind, which is a woody plant.

In some later work by Joshi (3) the yield of oats was determined where the whole plant and the leaves, stems, and roots of dhaincha, cowpeas, sann-hemp, and guvar were plowed under. In these studies, made in pot cultures and in plots in the field and extending over three years, the leaves were distinctly valuable in increasing the yields of both straw and grain. The stems and roots varied in their effect. In 20 cases, 13 yields were increased by the stems, 4 by the roots, and 2 showed neither loss nor gain.

The reduction in yield below the untreated soil in some cases with the stems and in the 14 cases with the roots could be ascribed to the low nitrogen content and the high cellulose content. This seems to be the logical explanation, since the whole plant was intermediate in its effect between the leaves and the stems, but of 20 comparisons the whole plant was ahead in 17 cases and equalled the stems in 1 case, leaving only 2 cases in which the whole plant was exceeded by the stems.

The effect of the stems and roots varied in the different experiments and years. This could probably be traced to the age of the plants or their maturity at the time of returning to the soil. The maturity would vary during different growing periods, and since the usaul period Joshi used was 8 weeks, this would enter as a large factor for such short season crops as some of these annual legumes.

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[1] Contribution from the Department of Agronomy, University of Illinois.

EXPERIMENTAL

The leaves, stems, roots, and a sample of the entire plant were analyzed for total nitrogen. These results are given in table 1 together with the dry matter in grams and the percentage of dry matter by weight of the various parts of the whole plant. The weight of nitrogen and its percentage of the total nitrogen contained in the various parts of the plant are also reported and will be referred to later in connection with a discussion of their nitrification. If the nitrogen content of the whole plant, as determined by an analysis, be compared with the calculated nitrogen content as obtained from the analysis of the parts, it will be seen that they differ by only 0.02 per cent.

TABLE 1

Dry matter and nitrogen content of leaves, stems, and roots of sweet clover

PART OF PLANT	DRY WEIGHT	PERCENTAGE WEIGHT OF ENTIRE PLANT	NITROGEN		
			Percentage	Weight	Percentage of total nitrogen in entire plant
	gm.			*gm.*	
Leaves	70	16.22	3.42	2.3940	21.31
Stems	104	23.74	1.35	1.4040	12.48
Roots	264	60.04	2.82	7.4448	66.21
Entire plant	438	100.00	2.50	11.2428	100.00

Fifteen milligrams of nitrogen in the form of the roots, stems, leaves, and the entire plant were placed in a typical silt soil, and nitrate determinations were made at intervals of 1 month throughout a period of 8 months. Table 2 shows the percentage of the total nitrogen nitrified in each 100 gm. of air-dry soil. A study of this table shows clearly the superior rate of nitrification exhibited by the roots as compared with the stems, leaves, and the entire plant during the first two months.

At the end of 3 months, the nitrification of the leaves had apparently reached 100 per cent and at the end of 8 months it had risen to 140 per cent. It appears that the leaves stimulated a greater nitrate production in this soil than the other parts of the plant and probably the real nitrification of the leaves would be less than 100 per cent. In spite of the large percentage of nitrification that occurred with the leaves, it is still evident that the rate of nitrification of the entire plant was controlled by the rate of nitrification of the roots.

Table 1 shows that the roots constituted 60.04 per cent of the dry matter by weight and contained 66.21 per cent of the total nitrogen present in the entire plant. The stems contained 23.74 per cent by weight of the dry matter but only 12.48 per cent of the total nitrogen of the entire plant, whereas the leaves represented only 16.22 per cent by weight of the dry matter, and but 21.31 per cent of the total nitrogen of the entire plant.

Calculating from these figures the amounts of nitrogen contained in the roots, leaves, and stems that entered into the 15 mgm. of nitrogen applied in the entire plant, one finds that the influence to be exerted by the roots would be five times that of the stems and three times that of the leaves, provided every other condition was identical. On the basis of the percentage nitrified in the parts, the percentage that should have been nitrified in the entire plant was calculated and is entered in table 3 as percentage calculated. By comparing these figures with the percentage nitrified of the entire plant actually found, it can not be said that the figures agree closely, although in some cases they approximate each other.

TABLE 2

Relative rate of nitrification of sweet clover roots, stems, leaves, and entire plant

(15 mgm. of nitrogen added per 100 gm. of soil)

PART OF PLANT	PROPORTION NITRIFIED							
	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Roots	37.3	82.7	69.3	83.6	80.0	64.4	90.7	91.6
Stems	0.9	0	8.4	16.0	17.8	35.6	71.1	80.0
Leaves	6.7	69.3	100.0	105.8	106.6	115.6	133.3	141.3
Entire plant	9.3	61.3	47.6	60.4	61.3	68.9	88.9	96.0

TABLE 3

Found and calculated values of percentages nitrified for entire plant

(15 mgm. of nitrogen added per 100 gm. of soil)

	PROPORTION NITRIFIED							
	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Entire plant found	9.3	61.3	47.6	60.4	61.3	68.9	88.9	96.0
Entire plant calculated	23.5	69.5	67.3	79.9	77.9	71.7	97.3	100.7
Entire plant calculated, leaves = 100	....	....	....	78.6	75.5	68.4	90.2	91.9

That the cause of the difference between the calculated and found percentages nitrified is in part due to the stimulation brought about by the leaves may be seen by the figures given for these same percentages if the leaves are taken at 100 only. These results prove the importance of studying the nitrification of the roots of legumes of this nature. It would be assumed at first thought that the fibrous nature of the roots would interfere considerably with their decomposition and this would also be assumed for the stems, but not for the leaves, while they were still in the green condition.

Relation of water-soluble nitrogen to rate of nitrification

In order to examine the cause of these differences in rate of nitrification, the water-soluble nitrogen in the parts of the sweet clover and in the entire plant were determined by leaching the same weights of samples as used in the experiment and determining the nitrogen obtained in the first 25 cc., second 25 cc., and a third portion of 50 cc. These results are reported in table 4.

This table shows that 86.6 per cent of the total nitrogen contained in the roots is water-soluble, whereas only 37.3 and 33.3 per cent respectively are water-soluble in the stems and leaves. The entire plant shows 67.3 per cent of total nitrogen as water-soluble. This figure for the entire plant is very close to the calculated value as obtained from the percentages of water-soluble nitrogen in the roots, stems, and leaves.

TABLE 4

Soluble nitrogen in parts of sweet clover tested for rates of nitrification

PART	WEIGHT	WATER-SOLUBLE NITROGEN					NITROGEN SOLUBLE IN NORMAL SALT SOLUTION	
		First 25 cc.	Second 25 cc.	Third 50 cc.	Total	Proportion of total nitrogen soluble	Total	Proportion of total nitrogen soluble
	gm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*
Roots	0.532	10.0	2.1	0.9	13.01	86.6	12.3	82.0
Stems	1.111	3.1	1.4	1.1	5.6	37.3	5.4	36.0
Leaves	0.435	2.1	1.6	1.3	5.0	33.3	....	....
Entire plant	0.593	5.5	2.3	2.4	10.1	67.3	9.2	61.3

It is shown here that the roots of a biennial legume contain about two and one-third times as much soluble nitrogen as the stems and about two and one-half times as much soluble nitrogen as the leaves, and that this additional soluble nitrogen would seem to be responsible for the much more rapid rate of nitrification of the roots during the first two months than either the stems or leaves yielded. At no time during the eight months period did the nitrate production of the stems equal that of the roots. The amount of nitrogen soluble in a normal salt solution is also reported in table 3 and shows only slight decreases as compared with the water-soluble nitrogen.

The presence of considerable water-soluble nitrogen in legume crops has been studied by several investigators. Osborne, Wakeman, and Leavenworth (5) reported that 44 per cent of the total nitrogen in fresh green alfalfa was water-soluble.

Kraus, Graber, Leukel, and Albert (4) at Wisconsin have found large amounts of the total nitrogen of alfalfa roots water-soluble.

In view of such findings and those reported by the authors, it is apparent that rate of nitrification studies must be considered in relation to the nature of the plant—whether annual, biennial, or perennial—the total nitrogen content, the fraction water-soluble, and the nature of the water-soluble fraction.

TABLE 5

Relative rates of nitrification of sweet clover roots and leaves in stirred and unstirred soil

(15 mgm. nitrogen added per 100 gm. of soil)

PART OF PLANT	PROPORTION NITRIFIED					
	1 month	2 months	3 months	4 months	5 months	6 months
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
			Stirred			
Roots	46.7	78.7	71.1	76.4	69.3	69.3
Leaves	13.3	61.8	97.8	103.1	113.8	120.0
			Unstirred			
Roots	37.3	77.8	66.7	70.2	65.3	60.9
Leaves	2.7	62.7	84.4	96.9	104.4	116.9

TABLE 6

Relative nitrification of sweet clover roots, stems, leaves, and entire plant

PART OF PLANT	WEIGHT OF NITRATE NITROGEN ON ACRE BASIS (WATER-FREE)							
	1 month	2 months	3 months	4 months	5 months	6 months	7 months	8 months
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Roots	174	297	309	348	360	350	354	350
Stems	88	111	172	196	220	285	310	324
Leaves	105	267	378	398	420	465	450	462
Entire plant	111	249	260	296	318	360	350	360
Soil alone	90	111	153	160	180	205	150	144

The water-soluble portion of the total nitrogen is believed to be responsible for the initial rapid rate of nitrification.

The water-soluble nitrogen must be present in relatively large amounts in order to drive the bacterial equilibrium toward nitrate production when high-carbon, low-nitrogen, or resistant carbon compounds are being studied. For example Barthel and Bengtsson (1) have recently reported a study of oat straw in sandy soil. The straw used contained 39.61 per cent of soluble nitrogen distributed as 0.154 per cent soluble protein, 0.116 per cent soluble amino nitrogen, and 0.02 per cent of ammonia nitrogen. This soluble nitrogen content was sufficient to permit the decomposition of the cellulose of the straw, but it was not sufficient for nitrification to occur during 4 months. Nitrates were produced when the nitrogen in the straw was reinforced with 42.4 mgm. of nitrogen as ammonium sulfate. This treatment contained 115.4 mgm. total nitrogen and 69 mgm. water-soluble nitrogen, and the ratio of C to N, assuming 38 per cent carbon in the straw, was 32 to 1. Even with such conditions prevailing, the nitrate accumulation was very small—only 3 to 3.7 mgm. per kilo at 2 months, and 16.5 to 18.4 mgm. at 4 months.

TABLE 7

Relative nitrification of roots and leaves and sweet clover in stirred and unstirred soil

PART OF PLANT	WEIGHT OF NITRATE NITROGEN ON ACRE BASIS (WATER-FREE)					
	1 month	2 months	3 months	4 months	5 months	6 months
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Stirred						
Roots	195	318	320	350	360	366
Leaves	120	280	380	410	460	480
Soil alone	90	141	160	178	204	210
Unstirred						
Roots	174	280	300	320	332	344
Leaves	96	246	340	380	420	470
Soil alone	90	105	150	162	185	207

Thus the present knowledge indicates that the water-soluble nitrogen fractions very largely account for the initial rapid nitrification but that they must be present in proportionally large amounts of the total nitrogen especially when cellulose in the form of mature non-legume crops is present in large amounts.

Relative rates of nitrification in stirred and unstirred soil

The rates of decomposition of the roots and leaves were tested in connection with another experiment and these results are reported in table 5, as they support the results given in table 2. A slight, consistent increase in nitrification was found as a result of occasional stirring of the soil. The pounds to the acre are given in table 7 and it is still more evident that the roots led the leaves the first two months and that stirring increased the nitrate formation.

CONCLUSIONS

1. The roots of the first season's fall growth of biennial white sweet clover although containing a smaller percentage of total nitrogen than the leaves, nitrified more rapidly than the leaves and stems.

2. The high water-soluble nitrogen content of the roots appears to account for the early rapid nitrification of the roots.

3. The rate of nitrification of the whole plant was dominated in the early stages by the roots because of the fact that they contained 66 per cent of the total nitrogen of the plant and because such a large proportion of this nitrogen was water-soluble.

REFERENCES

(1) BARTHEL, CHR., AND BENGTSSON, N. 1926 Decomposition of incrusted cellulose in soil. I. Straw and sawdust in clay and sand soils. Cent. Swedish Agr. Exp. Sta. Bul. 300. (Centralanst. för Försoksv. pa Jordbruksomradet Bakteriologiska andelningen No. 40.)

(2) JOSHI, N. V. 1919 Rate of nitrification of different green manures and parts of green manures and the influence of crop residues on nitrification. *Agr. Jour. India* 14: 395–413.

(3) JOSHI, N. V. 1922 Comparative manurial value of the whole plants and the different parts of green manures. Agr. Res. Inst., Pusa, Bul. 141.

(4) KRAUS ET AL. Unpublished data. University of Wisconsin.

(5) OSBORNE, T. B., WAKEMAN, A. J., AND LEAVENWORTH, C. S. 1922 The water-soluble constituents of the alfalfa plant. *Jour. Biol. Chem.* 53: 411.

(6) WHITING, A. L., AND SCHOONOVER, W. R. 1920 The comparative rate of decomposition of green and cured clover tops in soil. *Soil Sci.* 9: 137.

A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WIND-BLOWN SOIL: II. ATLANTIC COAST SAND DUNES, SANDWICH, MASSACHUSETTS

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INTRODUCTION

The present report is the second of a series of papers dealing with the bacteria of wind-blown soil. For a general discussion of the problem and a more complete list of references the reader is referred to the first report, of the series (5) which deals with Arizona soil, as the present paper will present, as briefly as possible, the results of a study of dune sand on the Atlantic coast.

METHODS AND MATERIALS

As a general rule the same methods were employed as in the first part of the work, consequently only deviations will be noted.

Collection of samples

The trench method was used for the collection of samples. From the two places selected for sampling, the soil was obtained with a brass soil-sampler at depths of 6, 12, and 24 inches from the surface. At each location the samples were taken across a foot-wide trench and placed in quart jars. The two samples for each depth were thoroughly mixed.

Study of the soil

The analysis of the soil was made in the same manner as was the arroyo soil except that a metal plate was used in the determination of combustible material.

Counting of plates and isolation of forms

Later work with the batch of plates from which those used in plating the 24-inch sample had been taken, showed that a few were not sterile, probably because of a too-closely packed hot-air sterilizer. Seven of the colonies fished from these plates were dim colonies at the bottom of the agar which, when cultured, proved to be lactose-fermenting spore-formers. These were considered almost certainly to be contaminations and were omitted from the list of isolated forms. The contamination was not great, and as the counts were all averages, and given in round numbers, they were considered within the range of error. Only those forms which grow aerobically on ordinary culture media are considered in this report. Counts are given to the nearest hundred.

Study of morphology

Part of the Gram's stains were made after five days' and part after two weeks' incubation at room temperature.

Culture reactions

The test for fermentation of glucose was made in broth, and of sucrose, in gelatin. Litmus milk was used instead of Purple Milk and was made by adding distilled water and litmus solution to skim-milk powder. Nitrate broth was made by adding 0.2 per cent of KNO_3 to Difco Dehydrated Nutrient Broth.

EXPERIMENTS AND RESULTS

Study of the soil

Situation. The soil was collected on September 12, 1924 from two slopes of a blow-out in the dunes at Sandwich, Massachusetts (plate 1, fig. 1). The blow-out ran approximately north and south and the slopes selected faced very nearly northeast and northwest (plate 1, fig. 2).

Rainfall. As no data were available for Sandwich the records for Hyannis, about 15 miles southwest of Sandwich, on the south shore of Cape Cod were used. These were obtained from the government weather records (6).

Annual rainfall for Hyannis, Mass.

	inches
1920	50.48
1921	48.16
1922	40.34
1923	39.63[1]
1924	39.08
Average	43.54

It is interesting to note that, although there had been a gradual decrease in precipitation during these 5 years, the average annual rainfall for 29 years (1892–1920 inclusive) was 43.69 inches, with a variation from 29.80 inches to 54.51 inches.

Size of soil particles. Table 1 shows the sizes of the soil particles for the three depths, determined July 31, 1925.

Water-soluble salts. A preliminary test by the author in August, 1925 showed such a slight difference in the three layers that the final analysis, in the spring of 1926, was made on a mixture of equal amounts of soil from the three depths. The results of this analysis are given in table 10.[2]

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[1] The record (31.95 inches) was for 10 months, with January and February omitted. In order to get an average annual rainfall for the five years the averages for January and February for the other four years were substituted for the missing figures.

Other Characters. A summary of other physical characters and of the hydrogen-ion concentration of the soil is given in table 2.

The numbers of organisms in the soil

In table 3 are given the results obtained from plates poured September 13, 1924, 24 hours after collection, and counted at the end of a week. As the number of colonies was rather small, it was planned to abandon these plates, hence they were not recounted at the end of ten days. It was impossible, however, to make a trip to the Cape to secure more sand. Also, according to Cutler et al (1), a second collection would probably yield different results. Consequently the same soil was plated at lower dilutions. These plates were too crowded to count at the end of a week; therefore, a third plating was made. There was an appreciable difference in numbers and proportions of forms after the two weeks' storage, consequently it was considered fairer to retain

TABLE 1

Weights of various sized particles in 100 gm. of soil

DEPTH	GREATER THAN 2 MM.	1–2 MM.	0.5–1 MM.	0.124–0.5 MM.	LESS THAN 0.124 MM.
inches	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
6	0	10.70	65.50	23.40	trace
12	0	1.80	72.20	25.90	trace
24	0	3.60	68.00	28.10	trace
All.........	0	5.37	68.57	25.80	trace

TABLE 2

Summary of the physical characters and of hydrogen-ion concentration of the soil

DEPTH	WATER CONTENT	WATER CAPACITY	PARTICLES BELOW 0.50 MM.	COMBUSTIBLE MATERIAL	RELATIVE WATER CONTENT*	OPTIMUM WATER CONTENT†	HYDROGEN-ION CONCENTRATION
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pH*
6	2.44	20.50	23.40+	0.20	11.90	14.35	6.8
12	2.56	25.00	25.90+	0.30	10.24	17.50	7.0
24	3.63	25.00	28.10+	0.30	14.52	17.50	7.2
All........	2.87	23.50	25.80+	0.27	12.21	16.45	...

* By relative water content is meant the percentage of capacity present.

† Optimum water content is taken to be 70 per cent of capacity.

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[2] The chemical analysis was made by Miss Miriam Dice, under the direction of Dr. Mary A. Griggs, Associate Professor of Chemistry, Wellesley College, to whom the writer presents her sincere thanks.

the first count. It is interesting to note, however, that although the actinomycetes remained practically the same, the molds increased and the bacteria and yeasts decreased, in general.

TABLE 3

Average total count and percentages of organisms in 1 gm. of fresh soil

DEPTH	DILUTIONS	NUMBER OF PLATES	AVERAGE TOTAL COUNT	ACTINOMYCETES	FUNGI	BACTERIA YEASTS
inches				*per cent*	*per cent*	*per cent*
6	1:1,000 1:10,000 1:100,000	14	199,900	28.25	2.95	68.78
12	1:1,000 1:10,000	8	49,400	14.97	1.82	83.40
24	1:1,000 1,10,000	8	31,900	13.79	1.56	89.63
All....................		30	93,700	24.01	2.56	73.53

TABLE 4

The percentages of colonies of various forms found on plates

DEPTH	NUMBER OF PLATES	TOTAL NUMBER	ACTINOMYCETES	FUNGI	BACTERIA YEASTS
inches			*per cent*	*per cent*	*per cent*
6	14	274	27.37	1.09	71.53
12	8	104	19.23	0.96	79.80
24	8	55	14.54	3.63	81.81
All.........	30	433	23.78	1.38	74.82

TABLE 5

Percentages of colored bacteria, or yeasts, on the plates

DEPTH	TOTAL NUMBER OF COLONIES	WHITE	YELLOW	ORANGE	RED	FLUORESCENT
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	89	91.01	0.00	3.37	1.12	4.49
12	113	64.60	7.96	0.88	0.88	25.66
24	116	75.00	12.07	0.86	0.86	11.20
All........	318	75.78	7.23	1.57	0.94	14.46

The proportions of the various organisms appearing on the plates were calculated in two ways. The number of colonies on the series of plates was counted, irrespective of the dilutions, and the percentages were calculated. These results are given in table 4. By the second method, the counts were reduced to number of organisms to each gram of soil and the percentages found. These results are given in table 3. Both methods are submitted because it is not always clear by which method the percentages reported by investigators were calculated. The colonies which lacked the distinguishing characteristics of actinomycetes or fungi were considered as "bacteria or yeasts," since it was impossible to distinguish between these two groups without a microscopic examination of each colony.

The numbers of colored colonies were counted on the third set of plates, irrespective of dilutions, and are shown in table 5. As is usual, the white forms predominated. Fluorescent colonies, which formed a large percentage of the total count on the third set of plates, were not noticed in the first set. On the other hand, the first set showed several brown forms. The fluorescent forms were almost without exception dim colonies on the bottom of the agar, showing color by reflected light. The fact that a large proportion of them occurred on three plates suggests the possibility of contamination. A comparison of the proportions of various forms and colors found among the cultures fished for study with those counted on plates, may be made by checking tables 4 and 5 with tables 6 and 7.

TABLE 6

Numbers of various forms fished for pure cultures

DEPTH	TOTAL NUMBER	ACTINOMYCETES	YEASTS	BACTERIA	UNDETERMINED*
inches					
6	34	1	6	22	5
12	36	0	8	26	2
24	16	1	1	13	1
All.........	86	2	15	61	8

* These forms died before their morphology was determined.

TABLE 7

Distribution of color in forms fished for pure cultures

DEPTH	TOTAL NUMBER	WHITE OR CREAM	YELLOW OR ORANGE	RED OR PINK	BROWN
inches					
6	34	18	11	5	0
12	36	25	7	3	1
24	16	8	7	1	0
All...........	86	51	25	9	1

The study of pure cultures

The majority of colonies were fished from the first set of plates. In several cases where dim, bottom colonies were used, the cultures were scrutinized very closely, especially those from the 24-inch soil. As has been stated, seven of these were discarded as contaminations.

Morphology. A summary of results of a study of stained material is given in table 8. It is quite evident from this table that the short rods predominated at 6 inches, the cocci at 12 inches, and the spore-bearing long rods at 24 inches.

Culture reactions. A summary of the culture reactions is given in table 9. Three forms which liquefied gelatin very slowly are classified with the non-liquefiers. A majority of the 17 cocci were gram positive and failed to ferment sugars, but digested gelatin. Of the 26 short rods, 1 was never obtained in pure culture, 12 were gram positive, only 3 fermented sugars, and 7 digested gelatin. The 18 long rods were mostly gram-positive spore-formers, which fermented sugars and digested gelatin. The non-spore-forming long rods were almost exclusively gram-negative, non-fermenters, but varied as to their power to digest gelatin. There was no gas formed in any sugar. There were, apparently, no dye-reducing forms in the upper layer. With two exceptions in the 12-inch layer, those forms which digested casein also reduced litmus.

TABLE 8

Summary of the morphology of bacteria in pure cultures

DEPTH	TOTAL NUMBER	COCCUS OR COCCOID	SMALL RODS	LONG RODS		GRAM'S STAIN	
				Spores	None	Negative	Positive
inches		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	22	13.63	68.18	4.54	13.63	57.14	42.86
12	26	38.46	34.61	19.23	7.69	61.54*	38.46
24	13	30.77	15.38	46.15	7.69	69.23	30.77
All........	61	27.87	42.62	19.67	9.83	61.66	38.34

* One variable.

TABLE 9

Summary of culture reactions after two weeks

DEPTH	BACTERIA CAUSING ACID FERMENTATION OF				BACTERIA CAUSING LITMUS TO FADE		BACTERIA DIGESTING				BACTERIA REDUCING NITRATES TO NITRITES	
	Glucose		Sucrose				Gelatin		Casein			
inches		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
6	5*	26.31	2	12.50	0	00.00	4	25.00	0	0.00	5	25.00
12	7	26.92	5	19.23	13	50.00	19	73.08	13	50.00	6	23.08
24	6	50.00	6	54.54	7	70.00	8	72.72	7	70.00	3	23.08
All.....	18	31.58	13	24.53	20	40.00	31	58.49	20	40.00	14	23.73

* The record for one form was neutral for glucose, acid for sucrose, and questionably acid for lactose. When the discrepancy was discovered, late in the year, the matter escaped attention until too late to repeat the experiment. The form is classed with glucose fermenters. This is the only case of even questionable fermentation of lactose, except in the reasonably sure cases of infection, previously mentioned.

DISCUSSION

In discussing the results here presented and comparing them with those obtained for Arizona soil, it must be understood that the work is in the nature of a survey. The very nature of the substratum—wind-blown soil—insures a constant change of material, and it is to be expected that analyses at intervals would show many variations. Consequently comparisons must be general, and small differences are not significant.

The annual rainfall at Hyannis, and presumably also at Sandwich, Massachusetts, is approximately 3½ times that at Tucson, Arizona. This not only affords a more frequently wet substratum, but also leaches salts from the soil. This is shown in table 10, in which a very brief summary for all depths at both localities is given.

TABLE 10

A comparison of the soluble salts and hydrogen-ion concentration for the two soils

SOIL	REACTION	TOTAL SOLUBLE SALTS	CO_3	SO_4	Cl	NO_3, ETC.
	pH	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Arizona	8.6–9.0	0.054	0.010	0.012	0.001	0.035
Massachusetts	6.8–7.2	0.007	0.005	trace	trace	0.002

TABLE 11

A comparison of the physical characters of the two soils

SOIL	WATER CONTENT	WATER CAPACITY	RELATIVE WATER CONTENT	PARTICLES 0.1 ± MM.	COMBUSTIBLE MATERIAL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Arizona	2	28	7	51	2
Massachusetts	3	24	12	trace	0.3

It is evident that the Massachusetts soil was much more nearly neutral than that of Arizona. In the more arid soil the upper layer was the most alkaline, whereas the reverse was true of the more humid soil of the coast. Salisbury (4) also found the pH of both frontal and the first high dunes to be about 7 at 4 inches from the surface, and to increase with depth. The amount of organic matter in Massachusetts dune sand agreed very well with his results but there seemed to be much less carbonate present both in Massachusetts dune sand and in Arizona arroyo soil than in the Blakeney Point dunes. Kelly (3) found the white (ammophila) dunes on the New Jersey coast to have a pH 6.8 from 15 cm. to 60 cm. This agrees with the pH of the 6-inch layer in the Massachusetts dune, but the pH did not remain constant with depth in the latter locality.

A comparison of the physical characters of the two soils is given in round numbers, for all depths, in table 11. The low water capacity, associated with the rather high percentage of fine material in the Arizona soil is puzzling. A great deal of time was spent in trying to get more accurate determinations. The small amount of the soil remaining had been poured together and an analysis of the mixture showed a decided loss of fine material in the process of repeated change of receptacles. Much time was spent trying the sedimentation method given by Emerson (2) and finally hundreds of soil particles were measured by eye-micrometer in the effort to obtain a more discriminating analysis. More soil was sent from Tucson,[3] from the exact place of the previous collection, but the character of the soil had changed in the interim; the sandy material had almost all disappeared, leaving the soil clayey in texture. A second analysis would not, therefore, be comparable, and it was not possible to check the original figures. From these figures the 12-inch soils are the most nearly alike in the two areas.

A comparison of the numbers of organisms found in the three layers of soil in the two situations shows that there were about eight times as many in the Arizona soil as in that of Massachusetts. The highest number was in the 12-inch layer in the former place and in the 6-inch layer in the latter locality. If the percentages of different forms be compared for all depths, the Massachusetts soil contained only three-fifths as many actinomycetes but twice as many fungi as the Arizona soil, whereas the percentage of bacteria and yeasts was only slightly greater in the former locality. The surprisingly large number of actinomycetes in the arid soil was noted in the previous paper. The fact that they increased in the lower layers instead of rapidly decreasing, as in Massachusetts, is interesting, and not clear from the facts in hand. Yeasts were present in both soils. The pure cultures fished from Massachusetts soil yielded, upon study, over twice as many as those from Tucson soil. Exclusive of the puzzling fluorescent forms previously mentioned, chromogenesis was very similar in the two areas, and yellow was probably the most common color. No blue, purple, or black colonies appeared on plates from either soil.

The only noteworthy difference in morphology is that, in proportion, Massachusetts soil had about twice as many short rods and about half as many long, spore-bearing rods as that of Arizona. The cocci and long non-spore-bearing rods were about equal in the two areas. Both soils had about twice as many gram-negative as gram-positive forms. In Arizona they were chiefly in the upper layer and in Massachusetts in the lower stratum.

There was greater digestive power in the bacterial flora of Tucson, chiefly because of the small percentage of digesters in the top layer of the Massachusetts soil. Otherwise the physiological activities were about equal in the two areas. In both soils the sugar fermenters increased in the deeper layers and there was a total lack of gas producers and a total, or almost total, lack of lactose fermenters.

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[3] The fresh supply of soil was collected and shipped by Mr. Godfrey Sykes of the Desert Laboratory of the Carnegie Institution of Washington, Tucson, Arizona, to whom the writer presents her thanks.

SUMMARY OF THE RESULTS OBTAINED FROM MASSACHUSETTS DUNE SAND

1. The average annual rainfall for 5 years previous to the collection of the samples was 43.54 inches.
2. The physical character of the soil was quite uniform. The upper layer was a little coarser and drier and poorer in organic matter.
3. Chemically the soil was almost exactly neutral and contained very little water-soluble salts. The most abundant of these were the carbonates.
4. Organisms were most abundant in the 6-inch layer and decreased downward.
5. The average percentages of the three groups of organisms were as follows: actinomycetes 24 per cent, fungi 1 per cent, and bacteria and yeasts 75 per cent.
6. White forms predominated. Yellow, orange, red, fluorescent, and brown forms were present. No blue, purple, or black colonies appeared on the plates.
7. Short rods predominated, followed in order of abundance by cocci, spore-bearing long rods, and non-spore-bearing long rods.
8. There were almost twice as many gram-negative as gram-positive forms.
9. No gas was formed in sugars, and there were only a few questionable cases of lactose fermentation.
10. Glucose and sucrose were fermented by one-third and one-fourth of the forms respectively.
11. One-half of the forms digested casein and reduced litmus, and two-fifths digested gelatin.
12. About one-fourth of the forms reduced nitrates.
13. A comparison is drawn between these results and those obtained from arroyo soil from Tucson, Arizona.

REFERENCES

(1) Cutler, D. W., Crump, L. M., and Sandon, H. 1922 A quantitative investigation of the bacterial and protozoan population of the soil with an account of the protozoan fauna. *Phil. Trans. Roy. Soc. (London) Ser. B.* 211: 317–350.

(2) Emerson, P. 1925 Soil Characteristics. New York.

(3) Kelly, A. P. 1925 Soil water of the New Jersey coast. *Ecology* 6: 143–149.

(4) Salisbury, E. J. 1922 The soils of Blakeney Point: A study of the soil reaction and succession in relation to the plant covering. *Ann. Bot.* 36: 391–431.

(5) Snow, L. M. 1926 A comparative study of the bacterial flora of wind-blown soil: I. Arroyo bank soil, Tucson, Arizona. *Soil Sci.* 21: 143–161.

(6) United States Department of Agriculture, Weather Bureau. 1920–1924 Climatological Data. New England Section, v. 32 (13); v. 33 (13); v. 34 (13); v. 35 (13); v. 36 (13).

PLATE 1

Fig. 1. Sand dunes at Sandwich, Massachusetts, looking eastward. This shows the general character of the area.

Fig. 2. The blow-out, or passage made between dunes by wind from Cape Cod Bay. The picture was taken looking approximately north. The collections of soil were made from bare regions on the two sides of the hollow.

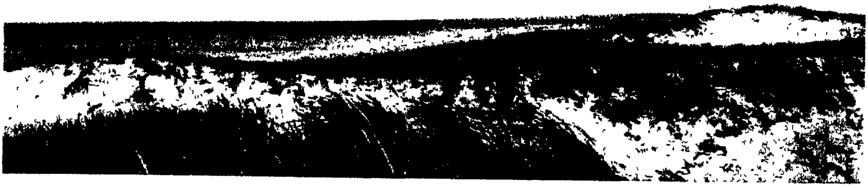

FIG. 1

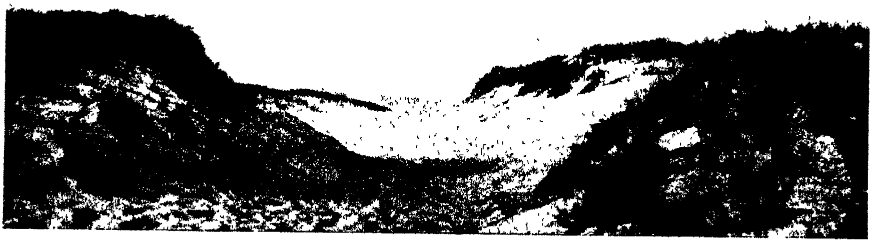

FIG. 2

LYSIMETER STUDIES[1]

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Continual or intermittent leaching of soil causes a gradual change in its condition as a result of the loss of certain elements important to the maintenance of fertility, tilth, and soil structure. Soil which is subject to leaching over long periods of time is deprived especially of its bases, which results in the development of acidity or base unsaturation.

The loss of plant-food nutrients has been studied by several workers using tanks containing soil so arranged that the water percolating through could be collected and measured for analysis. Similar studies covering a three-year period are briefly reported here.

LYSIMETER METHODS

Early in 1920, eight concrete tanks were constructed and filled with soil. Four of the tanks were filled with Willamette silt loam, a soil of fairly heavy texture though open in structure, having well developed drainage and good productivity. The remaining four tanks were filled with Dayton silty clay loam, a heavy soil with restricted drainage, low productivity, and a characteristic compact layer below the surface horizon. Soil from each 6-inch layer was kept separate and packed in the tanks in its natural position and volume.

In each series one soil tank is used as a check, a second is treated with lime, a third is treated with lime and manure, and the remaining tank is treated with manure alone. The rate of manure application is 20 tons to the acre and that of lime is 3 tons.

In the fall of 1921, the year the tanks were filled, each tank was planted to oats. Since then the cropping history has been barley, clover, barley, vetch, and vetch.

The soil was allowed to settle for a period of three years before chemical determinations of the plant nutrients were made. This was done in recognition of the fact that the breaking up and aeration of the soil affect the amount and composition of the percolate.

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[1] Abstract of thesis presented in partial fulfillment for the Degree of Master of Science.

[2] The writer wishes to express his appreciation for the helpful suggestions and criticisms given by Dr. W. L. Powers, under whose direction this work was conducted, and to Dr. R. E. Stephenson for assistance in analytical methods and helpful criticisms in preparation of this manuscript.

AMOUNT OF PERCOLATE

The amount of percolate received from the lysimeters depends upon amount and distribution of rainfall, crop grown, wind, temperature, soil type, treatment, and other factors. The yearly average rainfall for Corvallis is 42.4 inches, of which 45 to 77 per cent percolates through the soil, depending largely on the distribution of the precipitation.

The more open Willamette soil gives a greater loss of percolate than does the heavier silty clay loam. Applications of lime and manure to these soils cause a slight increase in the amount of percolate. Treatments of manure give the largest percolation loss. The increased loss of drainage water due to such materials results from increasing the porosity of the soil and from improvement of the soil structure.

COMPOSITION OF THE PERCOLATE

During seasons of light and well distributed rainfall, the first few liters of percolate in the season may represent fairly closely a displaced soil solution. The early rainfall causes swelling of soil particles and closing of channels. Later precipitation causes displacement of the soil solution which may compare favorably in composition with that obtained in the laboratory displacement on the same soil type.

Willamette Silt Loam

	N	Ca	SO_4	P	K
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Displaced soil solution[3]	trace	45.0	17.0	4.0	11.0
Percolate	21.8	36.5	11.5	1.3	11.4

The concentration of the percolate decreases in total parts per million as the leaching period advances, until the lowest concentration of the season is reached during the month of February.

REMOVAL OF CALCIUM

Calcium is lost in amounts greater than any other plant-food element. The check tank of the heavy Dayton soil gives an outgo equivalent to 71.1 pounds yearly loss of calcium, due to leaching. The amount of calcium lost is less than that observed by Lyon and Bizzell (2), McIntire (3), Hall, et al. (1), and others. This may result because the soils studied are low in calcium. Lime and manure treatments cause nearly double the loss of calcium when compared with the check tank, whereas treatments of lime alone and manure alone cause increases only during the percolation period following such application.

[3] POWERS, W. L. Studies with sulfur in relation to the soil solution. Unpublished.

TABLE 1

Loss of nutrients in percolate

YEAR	WILLAMETTE SILT LOAM				DAYTON SILTY CLAY LOAM			
	Lysimeter Treatments							
	Check 1	*L + M* 2	*M* 3	*L* 4	*L* 5	*M* 6	*L + M* 7	*Check* 8
				*Nitrates N**				
1923–1924	25.52	41.52	41.34	39.44	21.04	40.00	29.56	22.76
1924–1925	25.88	62.48	53.76	62.30	63.36	56.32	64.96	57.36
1925–1926	26.60	56.7	44.28	33.60	32.32	40.48	33.40	26.04
				*Calcium Ca**				
1923–1924	55.56	69.40	58.12	44.40	32.76	35.12	49.20	65.84
1924–1925	61.58	115.96	113.04	113.96	90.16	83.84	134.84	96.76
1925–1926	81.24	202.88	101.68	160.84	162.12	97.20	234.60	50.92
				*Sulfates SO_4**				
1922–1923	7.75	42.33	58.56	22.88				
1923–1924	13.40	25.36	31.12	42.12	37.24	58.82	92.40	40.12
1924–1925	49.80	116.88	109.36	90.36	92.56	120.92	106.32	90.48
1925–1926	25.56	558.92	45.48	263.56	468.00	32.80	360.80	33.04
				*Phosphorus PO_4**				
1925–1926	4.80	2.00	2.28	1.60	1.60	1.24	2.68	3.60
				*Potassium K**				
1923–1924	10.40	12.92	14.24	10.90	11.16	14.48	13.80	9.96
1924–1925	23.68	48.88	35.80	32.16	40.24	45.60	34.36	26.24
1925–1926	13.16	33.92	23.76	27.04	26.24	27.16	24.96	15.48
				*Magnesium Mg**				
1925–1026	21.20	41.88	22.60	38.68	23.28	30.32	30.32	23.40
				Total Leachings†				
1922–1923	12.67	24.06	28.63	27.64				
1923–1924	6.90	12.61	17.10	14.54	12.03	16.50	16.36	13.34
1924–1925	20.55	39.18	37.87	37.05	34.02	36.08	35.40	33.62
1925–1926	12.85	17.42	17.56	16.21	16.00	15.70	17.12	16.20

* Pounds to the acre.

† Inches.

L = lime. *M* = manure.

REMOVAL OF NITROGEN

Nitrates are lost from the two soils at the rate of 25 to 35 pounds from each acre annually when the soils are cropped. The Willamette loses more nitrogen than the Dayton soil. The lime and manure treated tank of this series lost 53.5 pounds of nitrogen from each acre. Treatments on the Dayton soil produce a smaller loss of nitrogen than do the same applications on the Willamette soil. Miller (7) reports a loss of 31.4 pounds of nitrogen from each acre; Lyon and Bizzel find a smaller total loss; whereas McIntire reports a greater loss of nitrogen from lime treated tanks (6). Nitrates are found in large quantities during the fall season when the first leachings are received. The amount decreases gradually until February when it reaches its lowest concentration.

REMOVAL OF SULFATES

The amount of sulfates lost from the check tanks seems to vary quite regularly with the total rainfall. The check tank of the Dayton series gives a 3-year average loss of 54.5 pounds of SO_4 from each acre. Applications of manure and lime materially increase loss of this element. The heaviest loss of sulfate occurs following application of fertilizing materials. In case of the lime treated tank of the Dayton soil and the lime and manure treated tank of the Willamette soil, the loss is more than 10 times the amount normally removed. The loss of sulfate increases to somewhat the same extent as the calcium loss, indicating that a portion at least is lost as dissolved calcium sulfate. McIntire (4) and Lyon and Bizzel (2) report much the same loss of sulfate as is found at this station. Sulfate is supplied to the soil at the rate of 3 to 10 pounds to the acre in Western Oregon rainfall, which therefore falls far short of compensating the loss by leaching.

REMOVAL OF POTASSIUM

The postassium loss is not large when compared to the great supply in the soil. The two soils lose much the same amount of potassium each year. An average annual loss of 15.7 pounds from the check plot of the Willamette soil is only slightly more than that from the heavier Dayton soil. Applications of of lime and manure increase the loss on the average about 10 pounds from each acre. The increase in potassium lost by leaching from the lime treated tanks is in direct contrast to that recorded by McIntire, Shaw, and Young (5), but agrees well with results obtained by Peck (8).

REMOVAL OF PHOSPHATE

Phosphate has been found in only minute quantities in drainage waters, as reported by other workers. At Rothansted, a mean-annual loss of 2 pounds from each acre is reported. The check tanks here reported lost 4 pounds of

PO_4 from each acre, as a result of one year's observation. Lime and manure treatments alone and in combination depressed the loss of phosphate to less than half that of the check plot.

REMOVAL OF MAGNESIUM

This base is lost from the check tanks in about the same chemical equivalents as potassium. The rate of loss through leaching is from 20 to 25 pounds from each acre yearly. Lime and manure treatments on the Willamette series double the loss of this base when compared to the check tank. Lime seems to be the cause of this increase. A replacing of the magnesium by the calcium ion probably occurs in the soil. The manure treatment of the Dayton series increases the loss of magnesium 7 pounds to the acre.

GENERAL DISCUSSION

From these lysimeter studies it is noted that the Willamette silt loam soil loses more plant nutrients in the percolate than does the heavier compact Dayton silty clay loam. In spite of this increased loss, the Willamette silt loam is much the better soil, measured by crop producing power. The difference in loss of nutrients and crop producing power of these soils is probably due to differences in physical condition and in the nature of the colloidal complexes. The Dayton is a very heavy soil with a very high content of colloidal clay, which has been concentrated in the subsurface horizon by leaching.

Of the nutrients lost by drainage, nitrogen and calcium are the only ones likely to become limiting factors in crop production. The constant removal of calcium by leaching is probably accompanied by replacement with the hydrogen ion, thus resulting in a base unsaturated soil that is acid. Finally such reactions may result in a calcium-poor soil, which will be of low production until lime is applied.

Such humid soils may be protected by cover crops or managed so that a minimum loss of bases and nitrogen occurs. Occasional applications of some of these elements, however, may be necessary to keep up permanent fertility.

SUMMARY

1. Under moderate weather conditions the first percolate approximates a displaced soil solution in chemical composition.
2. Applications of lime and manure, alone and in combination, increase the loss of total leachings and the plant nutrients, Ca, Mg, N, SO_4, and K.
3. The calcium ion is lost in larger amounts than any other one nutrient. An average of 65 to 70 pounds is lost from each acre each year.
4. Nitrates are lost at the rate of 25 to 30 pounds from each acre each year. Lime and manure treatments nearly double the amounts lost by leaching.

5. Normally sulfate is lost at the rate of about 50 pounds from the acre as SO_4. The loss is larger from tanks treated with lime the previous season.

6. Magnesium and potassium are lost at rates of 23 and 16 pounds from each acre each year. Lime and manure treatments increase the losses.

7. Phosphate is lost in small amounts from check tanks (4 p.p.m. of PO_4). Lime and manure treatments decrease the loss of this element.

REFERENCES

(1) Hall, A. D., Brenchley, W. E., and Underwood, L. M. 1914 The soil solution and mineral constituents of the soil solution. *Jour. Agr. Sci.* 6: 278.

(2) Lyon, T. L., and Bizzell, J. A. 1918 Lysimeter experiments. *N. Y. (Cornell) Agr. Exp. Sta. Mem.* 12: 1–115.

(3) McIntire, W. H. 1919 The carbonation of burnt lime in soils. *Soil Sci.* 7: 325–453.

(4) McIntire, W. H., Shaw, W. M., and Young, J. B. 1923 The influence of calcic and magnesic treatments upon sulfate leachings from a loam soil as measured by lysimeter leachings over an 8-year period. *Soil Sci.* 16: 1–40.

(5) MacIntire, W. H., Shaw, W. M., and Young, J. B. 1923 A 5-year lysimeter study of the supposed liberation of soil potassium by calcic and magnesic additions. *Soil Sci.* 16: 217–223.

(6) McIntire, W. H., and Young, J. B. 1925 Nitrate leachings as influenced by calcic and magnesic additions, with and without sulfur carriers. *Soil Sci.* 19: 309–323.

(7) Miller, H. H. J. 1906 The amount and composition of drainage through unmanured and uncropped land, Bamfield, Rothamsted. *Jour. Agr. Sci.* 1: 377–399.

(8) Peck, S. S. 1911 Lysimeter experiments. *Hawaii Agr. and Chem. Ser. Bul.* 37: 1–38.

REPLACEABLE BASES IN SOME OREGON SOILS

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Base exchange has been recently critically studied and its application extended to numerous and varied soil conditions. To date, however, no one has presented such data for the basaltic soils of the Pacific Northwest. Since the origin of these soils might prove to be responsible for a difference in their physical and chemical behavior, a study of their base exchange properties may be of interest.

The term "replaceable base" as used in recent work by Kelley (7), Scoffield (11), Gedroiz (3, 4), Hissink (5), and others refers to a rather definite exchangeable quantity of the cations of which Ca, Mg, Na, K, and NH_4 have been considered most important. More recently (6, 8, 9) the scope of the reaction has been extended to include, with a great deal of significance, the cation H, in connection with soil acidity problems. The cations, Al, Fe, and Mn also are of some importance in consideration of this latter problem.

Under proper conditions any one of these cations may replace at least part, and in many cases all, of the other cations which are held in the exchange complex. Exchange reactions of this type brought about under conditions of nature are responsble for deep seated soil changes. With limited rainfall, alkali soils may be produced; where rainfall is abundant, base-poor acid soils are produced; whereas with proper balance between rainfall and evaporation the tendency is to maintain soils rich in essential bases but about neutral in reaction.

Climatic conditions alone, however, perhaps do not in most cases rapidly bring about seriously harmful conditions. Thus the formation of alkali is often brought about more directly by seepage from higher areas or from poor drainage and a high water table, which favors the accumulation of harmful sodium salts that function in exchange reactions to produce alkali soils. Methods of farming and soil conditions in the humid areas which favor leaching, on the other hand, lead to the production of base-poor acid soils.

The development of methods for the quantitative measuring of the amount of the different replaceable bases, of replaceable hydrogen, or of the degree of saturation or unsaturation is of great importance agriculturally in both acid and alkali regions. The method of Kelley (6), for determining replaceable base has proved quite satisfacory. The acid hydrogen ion is more difficult to replace, and there is some question whether methods yet devised and published measure this cation on a quantitative basis.

In the study of California soils Kelley (7) has made three divisions; namely, normal soils which are non-acid and non-alkaline, alkali soils, and acid soils.

Oregon[1] has representatives of all the various groups of soils. Representing the acid soils, which are quite prevalent in the Willamette Valley, are the Waldo Silt Loam, Willamette Silty Clay Loam, Dayton Silty Clay Loam, and Carlton Silt Loam. The Antelope Clay Adobe and the Union Loam are from the section where neutral soils predominate. The Klamath Sandy Loam, Deschutes Sandy Loam, Hermiston Sandy Loam, and the Madiera Sandy Loam of California are representative of areas where alkaline soils frequently occur though none of these are more than slightly alkaline.

TABLE 1

Replacable bases as percentage of dry soil

SOIL USED	Ca	Mg	K	Na	pH
1. Waldo Silt Loam	0.0357	0.0182	0.0095	0.0255	4.8
2. Willamette Silty Clay Loam	0.3654	0.0016	0.0102	0.0381	6.5
3. Willamette Silty Clay Loam	0.2176	0.0230	0.0089	0.0110	6.5
4. Dayton Silty Clay Loam	0.1057	0.0122	0.0095	0.0501	5.7
5. Dayton Silty Clay Loam	0.2496	0.0284	0.0024	0.0149	
6. Carlton Silt Loam	0.2000	0.0113	0.0041	0.0122	6.1
Dry Farm Section					
7. Antelope Clay Adobe	0.8328	0.1827	0.0062	0.0303	7.2
8. (Swampy) Union Loam	0.6464	0.1278	0.0125	0.0026	6.5
Arid Soil Section					
9. Klamath Sandy Loam	0.1419	0.0093	0.0095	0.0501	7.0
10. Deschutes Sandy Loam	0.1383	0.0045	0.0138	0.0401	7.0
11. Madiera Sandy Loam	0.0400	0.0025	0.0021	0.0167	7.3
12. Hermiston Sandy Loam	0.1248	0.0202	0.0041	0.0243	7.6

DISCUSSION OF RESULTS

The data in table 1 show soil 1 of the acid group to be relatively low in replaceable calcium. The soil has a very high lime requirement and field experiments have shown a ready response to limestone treatments. No doubt soils very low in replaceable calcium may need applications of limestone to supply lime as a plant-food for legume crops. Such soils according to Kelley (7) may be of low productivity until the lime deficiency is made up.

Soils 2 and 3 are representative of the good valley soils. They run noticeably high in replaceable calcium—several times that of soil 1. These latter soils also show relatively small response to lime in field work, but seem capable of sustained high production when given proper care.

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[1] The soils used in this study were suggested by Dr. W. L. Powers, as especially suitable for the purpose.

Soil 6 is only a fair upland formation, inclined to be wet and heavy, largely because of seepage from higher areas. It is, perhaps, not deficient in replaceable calcium, and only moderately acid. Soils 4 and 5 on the other hand, are rather old formations of the valley-filling series in which a very heavy subsoil interferes with successful production of crops. The content of silt and clay is high and the infiltration of finer material from the surface has resulted in a very heavy and close subsoil. The colloid content of this heavy layer, though no analyses are available, no doubt runs very high. The content of fine material is such that under field conditions the lower horizon never dries beyond the consistency of putty even in the driest summer. These soils run lower in replaceable calcium than soils 2 and 3. Soil 5, however, is well supplied with this element and physical conditions are probably dominating factors in determining production. In field work these soils show response to limestone.

TABLE 2

Reaction values

SOIL	Ca	Mg	K	Na	TOTAL
1. Waldo Silt Loam	17.81	14.96	2.43	11.09	46.29
2. Willamette Silty Clay Loam	182.33	1.32	2.61	16.57	202.83
3. Willamette Silty Clay Loam	108.58	18.91	2.28	4.79	134.56
4. Dayton Silty Clay Loam	52.74	10.03	2.43	21.79	86.99
5. Dayton Silty Clay Loam	124.55	23.34	0.61	6.48	154.98
6. Carlton Silt Loam	99.80	9.29	1.05	5.31	115.45
Dry Farm Section					
7. Antelope Clay Adobe	415.57	150.18	1.59	13.18	580.52
8. (Swampy) Union Loam	322.55	105.05	3.20	1.13	431.93
Arid Soil Section					
9. Klamath Sandy Loam	70.81	7.64	9.42	19.23	107.10
10. Deschutes Sandy Loam	69.01	3.70	3.45	17.53	93.68
11. Madiera Sandy Loam	19.96	2.06	0.54	7.26	29.82
12. Hermiston Sandy Loam	62.22	16.60	1.05	10.57	90.49

The replaceable potassium does not vary to any extent in the valley except in soils 5 and 6. There are some variations in replaceable sodium, but under humid conditions such variations have little significance. The replaceable magnesium seems to vary somewhat inversely as the replaceable calcium. The average amount of replaceable magnesium is very much less than the average amount of replaceable calcium.

Soils 7 and 8 represent the dry farm section of the state. Both soils run high in replaceable calcium and magnesium, which may be considered somewhat characteristic of good neutral soils. The amount of these elements is from 2 to 10 times the amount found in soils of the acid humid area. It is not essential, however, that magnesium should vary with the calcium. The amount of replaceable potassium and sodium is quite similar to that of the valley soils.

The soils from the arid section of the state and soil 11, which is from California, are all good soils. None of the soils, therefore, represent the condition of serious alkalinity. Apparently the good neutral or approximately neutral soils of the arid section do not differ widely from those of other sections. The replaceable calcium, is however, lower than in the dry farm section, and somewhat lower than in the best valley soils.

Table 2 is a general summary expressing the various cations in equivalents with totals summarized in the last column. The soils of the acid group seem to run lower in total replaceable bases than do the soils of the neutral section. This is markedly true of the most acid soil. Since calcium is the base of dominant importance in acid soils, as well as perhaps in all good soils, it is logical to expect a heavy replacement and loss of this cation in the development of acidity. It is perhaps more important to maintain an ample supply of calcium than of any ion in the exchange complex. Either the exchange of calcium for hydrogen, resulting in acidity, or the exchange of calcium for sodium, resulting ultimately in serious alkalinity, is quite undesirable.

The soils from the arid section run rather consistently lower in total replaceable bases than from any section, perhaps largely due to the texture of the soils. The soils of this group are all sandy loams and are consequently low in colloidal material, which functions in absorption and exchange reactions. Both soils from the dry farm area on the other hand run high in colloidal material, as one is adobe and the other high in organic colloids. To this extent there seems to be a correlation between soil type and total replaceable bases.

In every case except soil 1, which has a lime requirement of nearly 6 tons by the Burgess (2) modified calcium acetate method, calcium is equal to more than 50 per cent of the total replaceable bases. By leaching this latter soil with 2 per cent calcium acetate until no more titratable acidity was removed it was possible to remove acid equivalent to 0.296 per cent of calcium. In other words, it is possible in this soil to replace hydrogen equivalent to nearly 8⅓ times the replaceable calcium that now remains, a condition which, no doubt, explains its ready response to applications of limestone. No other soil used shows the high acidity or the marked field response to liming.

GENERAL DISCUSSION

A casual consideration of the question might perhaps lead to the conclusion that there should be no definite amount of any of the cations that exist in replaceable form. All soil minerals are soluble to some slight extent and many perhaps participate in replacement reactions to some slight extent.

Present information, however, indicates that there is a more or less definite fraction of the soil variously denoted as the "absorption complex," "zeolitic" fraction from its supposed similarity to the zeolite minerals, "base exchange silicate" fraction, "exchange complex," etc. At present the exchange complex apparently is identical with the colloidal fraction in that it is part of the colloidal complex of soils. It is unnecessary, perhaps, to distinguish between organic and inorganic complexes as seat of the exchange reaction, for both are colloids and both are responsible for exchange reactions.

It is probable, however, that organic colloids are especially effective in conserving calcium, as calcium humates are relatively insoluble. Compounds of humus with sodium, potassium, and ammonium on the other hand are soluble and are either leached or help to destroy the desirable crumb structure of the soil. Calcium in organic combination therefore is not only itself conserved but helps also to conserve organic matter and to maintain a satisfactory physical condition in the soil.

Since colloids are much more reactive than is the coarse mineral portion of the soil, except where carbonates or other accumulations interfere, the coarse minerals may be practically disregarded in any immediate reaction. The first reaction in the decomposition of the native soil mineral which represents source material for the formation of soil colloids is, no doubt, one of hydrolysis, accompanied by either peptization or flocculation of the colloids thus formed.

A mineral fragment as it exists in the soil, normally is angular in shape, very hard, and very slightly or slowly soluble. The soil colloid on the other hand exhibits no definite shape or form, has no characteristic hardness, and is of relatively high solubility. Though a colloid in a mass represents great molecular size and complexity, the same colloid in the soil solution no doubt, represents a high degree of peptization and dispersion. It is this property of the colloid of passing into a highly dispersed form that gives it its relatively high reactivity. That much of the mineral portion of the soil probably has a protective coating of colloid, is an additional reason for considering the colloidal fraction of the soil as the source of the major immediate reactions effecting the supply and replenishment of ions in the soil solution, whether by exchange reactions or by direct solution.

The total quantity of exchangeable base in the soil therefore should represent the total base in certain colloidal combinations, and the quantity should show a rather constant value when replaceable hydrogen is included as one of the replaceable cations. At least the quantity value for replaceable base should be as constant as the total amount of colloidal material, which, it may be presumed, varies little from year to year, except as very long intervals are compared.

The exact identity of the colloidal silicates of the soil has not yet been established. The terms "colloid" and "clay fraction" are somewhat, if not entirely, synonymous. Clay has long been considered a mixture of many different compounds. Bradfield (1) and others conclude that colloids are mixtures of silicates of considerable complexity together with colloidal aluminum, iron, silicic acids, and organic matter. In alkali soils the colloid fraction would doubtless include a goodly amount of sodium silicate and some sodium humate.

Renick (10) suggests that compounds belonging to the Leverrierite group are probably an active source of replaceable base. This group of compounds is clay-like in its characteristics. Different clays vary widely, however, in the amount of replaceable bases, no doubt because of variations in composition and of different degrees of unsaturation that may exist.

No one has yet reported a study of replaceable bases in colloids isolated from soils.[2] Study of analyses shows that colloids average much higher in essential cations than ordinary soils, and indicates how small a percentage of the total colloidal bases are replaceable. Neither has it been definitely decided whether the replaceable portion of the colloids differs chemically from the non-reactive portion. Possibly replacement may occur only on the surface of colloidal clumps. If so, total replaceable base should be a function of dispersion, which is possible. It seems more likely, however, that definite chemical composition is essential to the replacement reaction. Complete saturation is that condition in which the colloid holds all the replaceable base that it is capable of holding. When part of the bases have been replaced by hydrogen, the soil is unsaturated as to replaceable base to the extent of the hydrogen replacement.

It has been asserted by some investigators that the base exchange is a physical phenomena in which the exchangeable base is held by adsorption forces. Adsorption is conceded to be a surface phenomenon whether physical or chemical. Formerly adsorption was considered to be due entirely to physical forces. At the present time evidence indicates that it is largely if not entirely chemical, or that it is unnecessary to make a distinction between physical and chemical forces in such reactions. Ordinary soil minerals are attacked chemically only at the surface or interfacial contact with the soil solution. Such reactions go slowly for the most part no doubt. The colloidal particle on the other hand, because of very small size and infinite surface per unit weight while the reaction is limited to points of interfacial contact, goes on with relatively great aggregate rapidity, though the rate of reaction per unit area of surface may be the same as for coarse particles. It would seem, therefore, that the term "adsorption" gives little explanation for any reaction occurring in soils which may not better be explained upon a purely chemical basis. Truog (12), Bradfield (1), Kelley (8), and others have emphasized this point.

There is, however, no consistent relation between physical composition and the amount of replaceable base in any similar group of the above soils. The six acid soils are all noticeably heavy in texture, relatively high in content of clay and silt and presumably of colloids, and yet they vary widely in amount of replaceable base. The neutral soils are rather coarse in texture, and yet some of them carry more replaceable base than some of the acid soils. The relative totals of replaceable base, and the ratios of the different elements evidently depend much more upon exchange reactions, the substitution of hydrogen for basic ions, and leaching during past ages, than upon mechanical composition. This is markedly illustrated in comparing soils of arid, semi-arid, and humid conditions. The geologic origin and basement of the soil also is no doubt a quite important factor.

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[2] Since this article was written, a very excellent paper has appeared: ANDERSON, M. S., AND MATTSON, S. 1926 Properties of the colloidal soil material. U. S. Dept. Agr. Dept. Bul. 1452.

In the humid section, nature no doubt operates to renew worn soils by eroding and removing the base-poor acid surface, thus allowing new complexes to form with a renewed supply of bases. Much can also be done in the management of soils to prevent unnecessary leaching. Farmers find it urgent to grow cover crops, and thus protect the soil at all times from unnecessary loss. The increased use of fertilizers and soil amendments also protects the soil, when intelligently done.

To a much greater extent in arid sections is prevention desirable and perhaps more effective than "cure" for alkali troubles. The reclamation of the most seriously alkaline soils is not yet possible on a practical basis. The prevention of alkali formation in the now good soils, on the other hand, is practicable and advisable by proper drainage and subsequent soil management.

SUMMARY

1. Acid soils are likely to be low in replaceable calcium, because of exchange of hydrogen for calcium and loss of the latter element by leaching.
2. The heavier soil types probably have greater base exchange capacity, but not necessarily a larger amount of replaceable bases.
3. Neutral soils tend to run high in percentage of replaceable calcium, which is the dominant replaceable base in both amount and function in good soils.
4. Soil alkalinity results from exchange of sodium for calcium. Consequently alkali soils are abnormally high in exchangeable sodium, which causes the alkalinity.
5. Both soil acidity and alkalinity can be retarded, if not practically prevented by proper soil management.

REFERENCES

(1) Bradfield, R. 1923 The chemical nature of colloidal clay. Mo. Agr. Exp. Sta. Res. Bul. 60.

(2) Burgess, P. S. 1922 The reaction of soils in the field as influenced by the long-continued use of fertilizer chemicals. *R. I. Agr. Exp. Sta. Bul.* 189: 9.

(3) Gedroiz, K. K. 1916 The absorptive capacity of the soil and zeolitic bases of the soil. *Zhur. Opytn. Agron.* (*Russian*) 17: 472.

(4) Gedroiz, K. K. 1924 Soils unsaturated with bases. Method of determining in soils, the hydrogen present in absorbed condition. Soil requirement of lime as a neutralizing agent. *Zhur. Opytn. Agron.* (*Russian*) 22: 3–7.

(5) Hissink, D. J. 1923 Method of estimating absorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269.

(6) Joffe, J. S., and McLean, H. E. 1926 Colloidal behavior of soils and soil fertility: II. The soil complex capable of base exchange and soil acidity. *Soil Sci.* 21: 181.

(7) Kelley, W. P., and Brown S. M. 1924 Replaceable bases in soils. Calif. Agr. Exp. Sta. Tech. Paper 15.

(8) Kelley, W. P., and Brown, S. M. 1926 Ion exchange in relation to soil acidity. *Soil Sci.* 21: 289.

(9) Page, H. J. 1926 The nature of soil acidity. *Trans. Second Comn. Internatl. Soc. Soil Sci.* A: 232.

(10) Renick, B. C. 1924 Base Exchange in Ground Water by Silicates, as Illustrated in Montana. U. S. Geol. Survey Water-Supply Paper 520-D.

(11) Scofield, C. S. 1924 Measuring the potential alkalinity of irrigated soils. *Jour. Wash. Acad. Sci.* 14: 192.

(12) Truog, E. 1925 The colloidal chemistry of soils. *Colloidal Symposium Monog.* 3: 228.

A RAPID APPROXIMATE METHOD FOR DETERMINING SOIL ORGANIC MATTER

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In soil survey and extension work it is often desirable to have a more precise idea of the organic matter content of a soil than a mere guess based upon color and texture, although the importance of the knowledge or the care taken in securing a sample may not warrant a determination by the standard methods. The following approximate method may be carried out in 10 minutes and appears capable of furnishing useful results. It is based upon the well known wet combustion method for total carbon in soil, and upon the commonly accepted average of about 50 per cent carbon and 5 per cent nitrogen in the organic matter of the usual soil types.

SOLUTIONS REQUIRED

The solutions required are prepared as follows:

Saturated potassium dichromate in concentrated sulfuric acid. Pulverize 20 gm. of the pure salt, add slowly with constant stirring to 1 liter concentrated sulfuric acid. Let stand for a week or more, closely stoppered, until the insoluble matter has settled out, and decant the clear solution into a glass stoppered bottle for preservation. If needed at once, the solution may be filtered through asbestos on a Buchner funnel, but it may deposit chromic acid after filtration. The solution is about 0.35 normal in oxidizing power. It is not perfectly stable on standing, however, and a blank should be run occasionally.

Fifth-normal ferrous ammonium sulfate. Dissolve 39.3 gm. of good quality crystals in water containing 10 cc. concentrated sulfuric acid and make to 500 cc. The solution keeps very well if protected from the air, but should not be used if the normal greenish tint has changed noticeably.

Diphenylamine indicator. Dissolve 0.5 gm. in 100 cc. concentrated sulfuric acid; pour carefully into 20 cc. cold water. This is the formula in general use as a qualitative test for nitrate.

PROCEDURE

Weigh 0.5 gm. air-dried and finely crushed soil, transfer to a large dry Pyrex test tube (the stock size 150 by 25 mm. is convenient). Add from a pipette 10 cc. chromic acid solution, letting the acid run down the sides of the tube to wash down any particles of soil. Stir carefully with a thermometer and heat with constant turning and stirring over a low flame until the temperature reaches 175°C., which should require about 90 seconds. Cease heating at once. The heat in the glass will probably raise the temperature a little. Let the solution in the test tube cool 1 minute in the air, after which it is safe to cool Pyrex test tubes in water. Pour the cooled solution into 150 cc. tap water in a 400-cc. beaker, then fill the tube twice with tap water, emptying each tube full into the beaker. Add a few drops of the diphenylamine solution, which should produce a deep rich blue color, although the characteristic deep blue often appears only after the titration is started. Titrate with the iron solution until the pure blue fades to a muddy greenish blue, which is the proper end point.[1] Deduct the reading from that secured in a blank experiment without soil: the difference is equivalent to the organic matter in the sample, which reduced a part of the chromic acid. With 0.5-gm. samples high in organic matter, the latter is not always completely oxidized, even though some chromic acid remains. If only a cubic centimeter or so of the iron solution is required in the titration, a repetition of the procedure with a smaller sample is advisable.

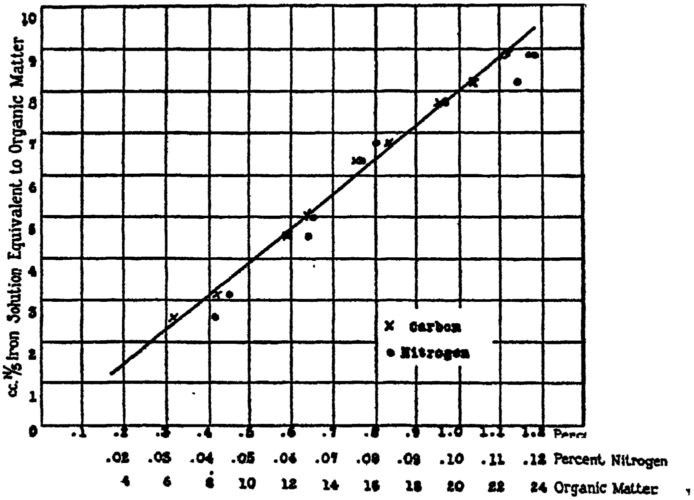

FIG. 1. GRAPH SHOWING RELATION BETWEEN REDUCTION OF CHROMIC ACID AND ORGANIC MATTER CONTENT OF 0.5 GM. SOIL

It is probably best for each operator to standardize his procedure and determine the value in organic matter of his solutions by a series of determinations on soils of known carbon content, of the same character as those with which he expects to work. The results secured may be plotted as in figure 1, and most of the calculations for subsequent determinations avoided, as the content of organic matter in 0.5-gm. samples may be read direct from the graph. If the sample in a particular case is not 0.5 gm. the result as read must be multiplied by the proper factor.

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[1] On the use of diphenylamine as an internal indicator for oxidation-reduction reactions, see Knop, *Jour. Amer. Chem. Soc.* 46 (1924): 263; Furman, *Jour. Indus. Eng. Chem.* 17 (1925): 314.

RESULTS

As an illustration of the results obtainable from this procedure when used with soils of the same type but varying in organic matter content, a series of 10 samples of surface soils representative of the entire range of several hundred samples from plots at this Station were run and the results plotted as shown in figure 1. In this figure, cubic centimeters of fifth-normal iron solution equivalent to organic matter in the sample (*i.e.*, blank titration minus titration with each 0.5 gm. sample) are represented by distance above the horizontal axis and percentages of organic carbon by furnace combustion corrected for inorganic carbon and of nitrogen by the Kjeldahl method, and estimated tons of organic matter to the acre 7 inches of surface soil, are represented by distances from the vertical axis. As may be seen, a straight line can be drawn to fall near all the points representing carbon contents, indicating that the procedure is of reasonable accuracy throughout the entire range of carbon contents. The points representing nitrogen contents do not always fall so near this line, which of course indicates that the carbon-nitrogen ratio of the soil is not a constant. The correspondence is close enough, however, to justify a rough estimate of the nitrogen content of a soil from this titration. The number of tons of organic matter in the surface soil of an acre is included although it was not determined directly, and is not represented by points.

TABLE 1

Known nitrogen and carbon content of soils and carbon content indicated by short method

SAMPLE	KNOWN CONTENT OF		INDICATED CONTENT OF CARBON
	Nitrogen	Carbon	
	per cent	*per cent*	*per cent*
Muck, 0.020 *gm*.	1.04	13.40	15.0
Fine sand, 0.300 *gm*.	0.11	1.33	1.3
Black clay, 0.100 *gm*.	0.31	3.53	3.6

With soils of widely varied types, as good results could scarcely be expected, but even so, the indications should be better than guesses. Several determinations of the carbon contents of very different soil types were made, and the results calculated from the graph in figure 1, multiplying by the proper factor to allow for difference in weight of sample. The carbon and nitrogen contents of these samples were known, and are given in table 1 with the carbon contents indicated by this short method.

These data indicate that even for soils of widely varying nature the method may still be useful without a special standardization for each soil type.

Highly abnormal soils, such as those containing much oxidizable mineral matter, could not be examined by this method, but such soils are probably rare.

A SUGGESTION FOR UNIFORMITY AND UTILITY OF DATA IN SOIL SOLUTION ANALYSES

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Comparison of analytical data representing soluble material in soils is often made involved because of the different methods of expression commonly employed. Thus, of the various elements or radicals under consideration, one may find parts per million, milligrams per hundred grams, milligram equivalents per hundred grams, etc. Not infrequently different forms of expression appear in the same article.

For all except total analyses of soils little need be said of the advantage obtained by expressing data in ionic form rather than in the form of hypothetical combinations or as the elements. Clarke (1) has fully discussed this point in connection with the statement of water analyses. It seems desirable, however, that a more workable statement of the amounts of radicals found should come into wider use.

A convenient method that adequately expresses the relations existing between the radicals found in a soil or soil solution is to be found in the criterion based on chemical value rather than on physical weight. This is the basis on which Stabler (3) established the practice of using "reacting values" in interpreting the results of water analyses. Reacting values are obtained by multiplying the reciprocals of the equivalent combining weights of the radicals by the amount of radical in milligrams per liter, and are, therefore, the same as milligram equivalents per liter. These terms, as Palmer (2) has pointed out, are peculiarly adapted to the chemical classification of waters, and their use permits more satisfactory and direct interpretation of analytical results than can be had from hypothetical combinations, or from statements of physical weight alone. They are similarly adapted for expressing the composition of soil solutions or percolates, and their use is recommended with the further suggestion that the basis of expression be made milligram equivalents per kilogram of water-free soil and designated as ***equivalent parts per million***.

The relation between parts per million and equivalent parts per million is shown by the formula.

$$(\text{Equivalent p.p.m.}) = (\text{p.p.m.}) \times \frac{1}{\text{Equivalent weight}}$$

Table 1 gives some equivalent weight reciprocals calculated from International Critical Tables atomic weights. A typical analysis stated in parts per million and equivalent parts per million for comparison is presented in table 2.

TABLE 1

Equivalent weight reciprocals of various radicals

POSITIVE RADICALS			NEGATIVE RADICALS		
	$\frac{1}{\text{Equivalent weight}}$	Logarithm*		$\frac{1}{\text{Equivalent weight}}$	Logarithm*
		-10			-10
(H)	0.9924	9.99667	(SiO_3)	0.0263	8.41987
(Fe'')	0.0358	8.55408	(PO_4)	0.0316	8.49928
(Fe''')	0.0537	8.73017	(CO_3)	0.0333	8.52288
(Al)	0.1113	9.04640	(HCO_3)	0.0164	8.21462
(Mn)	0.0364	8.56122	(SO_4)	0.0208	8.31846
(Ca)	0.0499	8.69821	(NO_3)	0.0161	8.20755
(Mg)	0.0822	8.91507	(Cl)	0.0282	8.45028
(Na)	0.0435	8.63833	(OH)	0.0588	8.76936
(K)	0.0256	8.40787			
(NH_4)	0.0554	8.74379			

* Calculated from the fraction $\frac{1}{\text{Equivalent weight}}$.

TABLE 2

Analysis of 1 normal NH_4Cl extract of Palouse silt loam soil

	Parts per million	Equivalent parts per million		Parts per million	Equivalent parts per million
(Fe''')	29	2	(SiO_3)	1272	33
(Al)	115	13	(CO_3)	0	0
(Mn)	40	1	(HCO_3)	868	14
(Ca)	13,710	684	(NO_3)	88	1
(Mg)	395	32	(SO_4)	200	4
(Na)	595	26			
(K)	475	12			

REFERENCES

(1) Clarke, F. W. 1924 The composition of the river and lake waters of the United States. *U. S. Geol. Survey Prof. Paper* 135: 2.

(2) Palmer, C. 1911 The geochemical interpretation of water analyses. U. S. Geol. Survey Bul. 479.

(3) Stabler, H. 1908 The mineral analysis of water for industrial purposes and its interpretation by the engineer. *Engin. News-Rec.* 60: 356.

THE MEASUREMENT OF "SUCTION FORCES" IN COLLOIDAL SOILS

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In a recent paper, Joffe and McLean (6) describe a method for estimating soil colloids by measuring their "suction force," employing for the purpose a special form of auto-irrigator furnished with a mercury manometer. After placing the auto-irrigator filled with water in a large vessel, and packing round it soil, presumably—though not stated to be—previously dried to a constant low moisture content, they observe the change in level of the mercury column of the manometer when equilibrium has been attained. They state that equilibrium is reached when "the layer of soil in contact with the porous container has become saturated."

Although Joffe and McLean's experimental data appear qualitatively to differentiate between sandy, loamy, and clayey soils, it is here submitted that their method cannot be expected to yield accurate quantitative results, because, in their theory, no account is taken of the water-conductivity factor in the moisture relations of soils.

The theory of the instrument used by Joffe and McLean is similar to that developed by Livingston for the porous porcelain soil auto-irrigator, the thistle osmometer (9), and the conical porous porcelain soil-point (7), and adopted by Mason for the writing-pencil soil-point (8). Livingston's theory is based essentially on a dynamic conception, and provides precise definitions of the terms "water-absorbing power of a dry soil," and its converse, "water-supplying power of a moist soil." These terms postulate a soil property whereby water is taken up from a free-water reservoir or delivered to an active absorbing surface at a definite *rate*, which is decided, not only by the magnitude of involved forces of absorption, suction, or "imbibition," but also by the resistance opposed by the soil to water movement. Thus, in the auto-irrigator system a *gradient* of soil moisture content is eventually established. This gradient may be "dynamic" if plants are actively growing in a soil-mass irrigated by the instrument, or it may be ultimately "static" if the soil-mass contains no plants and is hermetically sealed within an impervious container. The "steepness" of the moisture gradient in either case is probably chiefly decided by the conductivity of the soil for water. It is a gentle gradient in sandy soils of high water-conductivity, and a steep gradient in non-conducting colloidal soils whose composite particles cohere. In certain colloidal soils, the moisture gradient surrounding the irrigator vessel may conceivably fail to extend as far as the roots of plants situated some distance from the vessel, so that the instrument may not satisfactorily perform its functions. Hence, in controlled moisture studies of plants, the precaution is usually taken to employ soils of suitable water-conductivity for filling the containers into which auto-irrigator and plant are introduced.

Similarly, in the soil-point system, a gradient is also established, but the gradient in this case must be maintained in a dynamic condition by limiting the time of contact between soil-point and moist soil in comparative trials with soils in laboratory or field (8, 3, 4). Moreover, soil-points should be carefully standardized by bringing them to a definite degree of dryness before inserting them into moist soil (8).

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[1] The writer wishes to acknowledge valuable criticism and help received from Dr. Burton E. Livingston, of Johns Hopkins University, who kindly read the original draft of this paper.

In accordance with these ideas, the main objection to the method of Joffe and McLean would appear to lie in their failure to define the spatial limits of the "layer of soil" which becomes "saturated" by contact with the water-filled porous vessel. It is possible to conceive two sorts of colloidal soils exhibiting, when dry, identical suction forces, yet possessing different degrees of conductivity. Such instances are described below (see the next section). In the case of the colloidal soil of *low* water conductivity, the rate of water movement may be relatively very slow; the static moisture gradient very steep; and the dimensions of the saturation layer in contact with the porous container, very small. In the case of the colloidal soil of *high* water conductivity, the rate of water movement may be relatively high; the static moisture gradient (if ever established), quite gentle; and the dimensions of the saturation layer, appreciably large. Since the apparatus of Joffe and McLean gauges suction forces in terms of volume loss of water,[2] it is obviously would fail to demonstrate equal suction forces in the two hypothetical soils under consideration.

Joffe and McLean's application of their method to a study of the effects of fertilizers and lime on the magnitude of the suction force of soils, is open to criticism in that it also fails to eliminate the conductivity factor as influenced by the various treatments.

EXPERIMENTAL

Three colloidal soils of different types were kneaded with water in amounts sufficient to bring the soils to their points of stickiness. It is assumed that the point of stickiness of colloidal soils marks the condition when the colloidal component is just completely saturated with water (2). The three soils therefore were believed to be in comparable states as regards their water relations. Each of the kneaded soils was pressed into five cigarette tins which were immediately sealed with paraffin wax to prevent water loss by evaporation. The tins and contents were weighed on successive days to insure that the wax seals were acting efficiently. Standardized writing pencils, of the sort used by Mason (8) and by the writer (3) in studies of the water-supplying powers of soils, were inserted through holes made in the wax so as to establish contact with the kneaded soils near the central regions of the tins. Three weighed pencils were inserted into each tin of soil. A three-hour contact was allowed. The pencils were then removed and rapidly weighed with the usual precautions. The experiment was repeated next day by inserting another set of standardized pencils into fresh holes made in the wax seals, the former holes having previously been plugged with soft wax. Finally, the tins were emptied and the moisture contents of the soils determined by weighing and drying in the air-oven.

The results obtained are given in tables 1 and 2. The characteristics of the soils are presented in table 1, and their water-supplying powers in table 2.

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[2] It may be objected that Joffe and McLean's instrument gauges suction forces, not by water loss, but by reduction of a hydrostatic head (negative pressure). It is admitted that although the instrument must impose a gradually increasing resistance to the passage of water outwards, the maximum magnitude developed by this resisting force is not the only factor deciding retention of water by the instrument. The inability of the soil to take up and to store more than a certain limited amount of water in the zone of decreasing moistness surrounding the porous vessel, must also be a factor deciding ultimate *quantity* of water lost.

Discussion of experimental results

Although the soils exhibit similar degrees of colloidality, and contain approximately similar amounts of colloidal matter as indicated by their physical constants (particularly the moisture content at the point of stickiness), yet their water-supplying powers differ considerably. The lateritic soil appears to be the most colloidal of the three soils, and would therefore perhaps be expected to retain its moisture with the greatest force; yet its water-supplying power is the highest.

TABLE 1

Physical constants of the soils employed*

	SOIL 1—NON-CALCAREOUS SILICEOUS SOIL, ANTIGUA	SOIL 2—CALCAREOUS SILICEOUS SOIL, ANTIGUA	SOIL 3—RED LATERITIC SOIL BARBADOS
Mechanical composition, (approx. per cent silt and clay)	62	63	75
Calcium carbonate content	0.2	7.2	0.3
Organic matter content†	1.3	1.0	0.5
Volume expansion on wetting	66.9	60.8	17.2
Relative degree of cohesiveness‡	3.41	2.59	2.48
Relative settling rate	0.6	92.0	100.0
Hydroscopic coefficient	13.0	12.0	20.6
Moisture content at the point of stickiness	46.7	45.9	48.8

* Constants other than ratios are expressed as percentages on oven-dry (110°) material.

† Wet combustion method.

‡ See Hardy, F., 1925, *Jour. Agr. Sci.* 15: 420–433.

TABLE 2

Water-supplying powers of the kneaded soils, by Mason's method (8)*

(Hundredths of a gram of water absorbed by one soil-point in a three-hour contact)

	FIRST DETERMINATION	SECOND DETERMINATION
Soil 1—non-calcareous siliceous soil	1.72 ± 0.060	1.64 ± 0.066
Soil 2—calcareous siliceous soil	2.71 ± 0.041	2.45 ± 0.040
Soil 3—red lateritic soil	3.12 ± 0.039	2.76 ± 0.032

* The results, with their probable errors, are means of five separate determinations, each involving three soil-points. Their numerical differences are statistically significant in every

The differences in water-supplying power may be ascribed to differences in conductivity for water for the three soils, which are probably eventually decided by differences in *cohesiveness* (table 1) between the colloid-coated grains comprizing the plastic soil-masses. In the cases of the lateritic soil and the calcareous soil, the colloid coatings are not so well “run together” as in the non-calcareous soil, which exhibits highest cohesiveness and lowest rate of settling from suspension in water. Further data presented below support this contention.

EVALUATION OF SUCTION FORCES FROM PERCOLATION CONSTANTS

In their studies on percolation of water through soils, Green and Ampt (1) designate the force which tends to draw water from saturated to dry regions of a soil, per unit area of cross section of the pore spaces, as the *Capillarity Coefficient* (K). Green and Ampt's methods of examination enable one to estimate the magnitude of this coefficient for sifted dry soils packed uniformly in glass tubes.

The writer (5) has elsewhere employed the methods of Green and Ampt in a study of percolation in certain *colloidal* soils, including the three soils used in the present investigation. Assuming that, in dry colloidal soils, the forces producing water movement are mainly suction forces, or forces of water imbibition, rather than capillarity forces, it is possible to evaluate their magnitudes from the writer's data. The data and values are presented in table 3.

TABLE 3

Percolation constants of the soils employed

FINE SOILS, PASSING A 1 MM. SIEVE	SOIL 1—NON-CALCAREOUS SILICEOUS SOIL	SOIL 2—CALCAREOUS SILICEOUS SOIL	SOIL 3—RED LATERITIC SOIL
Distance traversed by water-front in first 10 hours.................................*cm.*	1.9	6.8	23.4
Value for C in formula $t = Cl^n$ (t in hours).......	2.67	0.35	0.017
Permeability constant, (P) × 10^4.............	0.001	0.059	0.470
Specific pore space (S)........................	0.433	0.454	0.590
Capillarity coefficient* (K) ×10^{-2}.............	2.25	0.30	1.02

* Calculated from the formula, $C = \frac{}{2PK}$; (time unit, the second).

Discussion of results

Although Green and Ampt's formulae were found by the writer not strictly to apply to highly colloidal soils, yet the data in table 3 certainly indicate that the three colloidal soils employed in the present investigation differ markedly in their water conductivities, the lateritic soil being the most conductive, and the non-calcareous siliceous soil, the least conductive. The calcareous siliceous soil occupies a somewhat intermediate position. The order of conductivities coincides with the order of water-supplying powers of the moist kneaded soils.

The values for capillarity coefficients are not very reliable, since they are derived from values of two other constants whose measurement is difficult and not very precise; particularly because in horizontal flow experiments, the granular material swells to different degrees (table 1) on progressive wetting. Nevertheless, the calculated values are approximately of the same order of magnitude, and, considered with the physical constants of the three soils, indicate that suction force is perhaps not the major factor responsible for the specific differences in the water-supplying powers of the soils. It is not considered, however, that Green and Ampt's method furnishes a reliable practical means of measuring suction forces in colloidal soils, but its fundamental theoretical basis appears to the writer to be less open to criticism than that underlying Joffe and McLean's auto-irrigator method.

SUMMARY

1. Joffe and McLean's method for measuring suction forces in soils (especially colloidal soils), is criticized on the grounds that it takes no account of variations in water conductivity of soils.

2. Experimental data, based on measurements of the water-supplying powers of the three different types of soils of approximately similar colloid contents, are adduced to demonstrate that water conductivity may exercise controlling influence over the distribution of water in soil surrounding Joffe and McLean's instrument when in position, and may therefore influence the measurements made.

3. Green and Ampt's method for evaluating suction forces (capillarity coefficients) is discussed and applied to the three soils studied. It is considered, however, not to be sufficiently precise as a practical method, although less open to criticism on theoretical grounds than Joffe and McLean's method.

REFERENCES

(1) Green, W. H., and Ampt, G. A. 1911 Studies on soil physics: I. The flow of air and water through soils. *Jour. Agr. Sci.* 4: 1–24.

(2) Hardy, F. 1923 The physical significance of the shrinkage coefficient of clays and soils. *Jour. Agr. Sci.* 13: 243–264.

(3) Hardy, F. 1923 The soil-point method for directly estimating the water-supplying power of a soil in the field. *Jour. Agr. Sci.* 13: 355–360.

(4) Hardy, F. 1924 Field measurement of soil moisture. *Trop. Agr. (Trinidad)* 1: 98–100.

(5) Hardy, F. 1925 Percolation in colloidal soils, considered in relation to swelling and cohesiveness. *Jour. Agr. Sci.* 15: 434–443.

(6) Joffe, J. S., and McLean, H. C. 1925 Colloidal behaviour of soils and soil fertility: I. Suction force of soils as an index of their colloid content. *Soil Sci.* 20: 169–174.

(7) Livingston, B. E., and Koketsu, R. 1920 The water-supplying power of the soil as related to the wilting of plants. *Soil Sci.* 9: 469–485.

(8) Mason, T. G. 1922 The physiological humidity of the soil and its direct determination. *West Indian Bul.* 19: 137–154.

(9) Pulling, H. E., and Livingston, B. E. 1915 The water-supplying power of the soil as indicated by osmometers. *Carnegie Inst. Wash. Pub.* 204: 49–84.

BOOK REVIEW

Lehrbuch der Enzyme, Chemie, physikalische Chemie und Biologie. By PROF. CARL OPPENHEIMER, with the collaboration of DR. RICHARD KUHN. G. Thieme, Leipzig, 1927. 660 pp., 18 figs. Price, unbound, M. 33; bound, M. 36.

The fifth edition of the well known threatise of Oppenheimer on Enzymes (*Die Fermente und ihre Wirkungen*) has reached such large proportions (over 2000 large size pages in the first two volumes that have just appeared, with two more to follow, that the idea has naturally presented itself to prepare an abbreviated edition, which could be used as a textbook by students of enzymes. The result is the book under consideration. This book is more than an abbreviated edition—some parts have been entirely rewritten, because of the very rapidly growing subject of enzymes. The voluminous literature of the Handbuch has been practically left out in the Lehrbuch, with the exception of the most recent contributions, the author constantly referring to the more extensive publications.

The book is divided into four parts: Part I contains chapters on the general chemistry of enzymes, including a description of them, the influence of various factors (physical, chemical, and specific activators and paralyzers) upon enzyme action, physical chemistry, and kinetics; Part II contains chapters on the biology of enzymes, including the occurrence and formation of enzymes, and the rôle of enzymes in life processes; Part III contains the chapters on hydrolases, including the esterases, carbohydrases, polyases (enzymes acting upon polysaccharides), nucleases, amidases, proteases and thrombas; Part IV includes chapters on the enzymes of decomposition or the desmolases, including a detailed discussion of the various theories of oxidation-reduction; namely, the peroxide theories, indirect oxidation, and hydrogen activation, also the enzymes of hexose-decomposition or the zymases, various dehydrases which are important in metabolism, including the dehydration of cyclic chromogens, and finally the catalases. This is followed by a subject index.

The descriptive part occupies over two-thirds of the book. In describing the individual enzymes, the author discusses in detail their chemical nature, specificity of action, occurrence, the influence of chemical and other factors, and preparation. The methods for measuring the action of the various enzymes are not considered sufficiently and in many instances are left out altogether. The application of enzyme action is usually not considered at all. This may be because the last two volumes of the large edition of the Handbuch, namely, those dealing with Methodics and Technology of enzymes, have not appeared as yet.

The book is well printed and is concise and readable. The discussion of the various theories of oxidation-reduction is especially interesting.

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ERRATA

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"The Microflora and the Productivity of Leached and Non-Leached Alkali Soil," by J. E. Greaves

The following percentages throughout this article should be changed:

Page 277, line 12, *20* should be *21*, *140* should be *53*
line 13, *67* should be *70*
line 14, *92* should be *80*
line 15, *30* should be *38*
line 17, *30* should be 20
line 19, *41* should be *60*
Last paragraph, line 2, *11* should be *20*
Page 278, line 3, *830* should be *210*
Page 280, paragraph 3, line 1, *12* should be *13*
Paragraph 4, line 1, *10* should be *11*
Page 284, paragraph 3, line 3, *10* should be *11*
Paragraph 6, line 1, *12* should be *21*
Page 287, line 2, *12* should be *21*
Page 295, paragraph 8, line 2, *190* should be *187*
Page 301, paragraph 4, line 1, *11* should be *20*
Paragraph 5, line 2, *830* should be *210*
Paragraph 6, line 1, *10* should be *11*
Paragraph 7, line 3, *12* should be *21*
Page 296–7, table 19, Subheadings *Roots* and *Tops* should be interchanged

NITRATE ASSIMILATION BY SOIL MICROÖRGANISMS IN RELATION TO AVAILABLE ENERGY SUPPLY[1]

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INTRODUCTION

The probability of failure to secure large increases in crop yields following the application of such materials as straw, hay, certain types of fresh manure, and various crop residues having a high nitrogen-carbon ratio has been repeatedly emphasized by agricultural writers and research workers. Frequently these materials have actually injured plant growth and depressed the yields. Hence, farmers have been freely advised not to apply large quantities of such materials just prior to planting but rather first to allow the materials to decay or to plow them under several weeks before planting. Even then the use of a crop such as corn, which thrives on decaying organic matter, has been advised.

The literature on the subject is so voluminous that it is not advisable to discuss even the leading articles on the subject in this paper. Suffice it to say that many of the earlier writers attributed the injuries to toxicity or to denitrification, using the term to mean in many cases a loss of nitrogen from the soil. The more recent investigators have, however, almost unanimously agreed that the bad effect of carbohydrate materials when added to soils is due to the fact that they serve as food for bacteria and thus greatly increase bacterial numbers and activities, particularly the ammonifiers and cellulose decomposers, which require nitrogen for food and hence use the readily available forms, particularly nitrates, present in the soil solution. They grow, therefore, in competition with higher plants and naturally the latter must suffer from partial nitrate starvation. The work reported in this paper was undertaken in order to obtain additional data on some of the least understood phases of the subject.

EXPERIMENTAL

The importance of the subject under discussion was first brought to the attention of the writer by some results obtained with oats grown in the greenhouse. Although reported elsewhere (1), these data are given here in brief form.

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[1] A joint contribution from the Fixed Nitrogen Research Laboratory, Bureau of Soils, United States Department of Agriculture, and the New Jersey Agricultural Experiment Station, the work having been started at the latter and completed at the former.

Paper No. 196 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

Effect of various sources of organic matter on the growth of oats

For this experiment, a rather poor Miami sandy loam soil was chosen. To pots holding 36 pounds of soil were added various sources of organic matter in a finely ground condition at rates approximating field conditions. The results reported in table 1 were obtained.

It will be observed that the materials having the widest nitrogen-carbon ratios; namely, oat straw, timothy hay, corn stover, and fresh horse manure, decreased the yield, whereas cow manure, rotted manure, clover hay, and cowpea hay, increased the crop yields. Since the same amount of nitrogen was not added in all cases, the yields can not be considered as strictly comparable but merely as indicative of the effects of the different materials. It should be stated further that the early injuries to the crop by the materials of wide nitrogen-carbon ratio were even greater than the yields indicate. The young oat plants, especially those grown in the presence of fresh stable manure, turned yellow and nearly died. There was a marked recovery later and the second crop was actually better on these pots than where the rotted manure had been used.

TABLE 1

Yields of oats from a soil fertilized with different types of organic materials

TREATMENT PER ACRE	FIRST CROP			SECOND CROP
	Dry weights	Relative weights	Nitrogen	Dry weights
	gm.		*per cent*	*gm.*
Check	60.1	100.0	0.73	7.1
15 T. horse manure	31.2	51.9	0.84	12.2
15 T. cow manure	66.8	111.1	0.73	13.9
15 T. rotted manure	87.1	144.9	0.79	8.3
2.5 T. oats straw	30.7	51.1	0.77	11.1
3 T. corn stover	51.3	80.4	0.79	10.8
2 T. timothy hay	45.0	70.4	0.71	9.1
4 T. cowpea hay	72.6	120.8	0.89	10.6
4 T. clover hay	63.8	106.0	0.82	9.5

From the experiment reported above, as well as from results given in the literature, the writer was convinced that the injuries produced by fresh stable manure were of exactly the same nature as those caused by similar sources of energy, such as straw, sugar, and mature corn stalks. Because of the great importance of manure in agriculture and also because it has been studied to a lesser extent as an energy source than some of the other materials, particularly straw, it was decided to limit these studies to this material, with the fairly certain knowledge that quite similar results would have been obtained if sugar or straw had been chosen. In the experiments which follow, wherever a manure is mentioned the reader will know that, unless otherwise specified, the term refers to fresh solid horse manure, dried and sterilized in order to eliminate the biological factor.

Effect of fresh manure on soil nitrates

This experiment was carried out at the beginning of these investigations in order to obtain further proof of the now generally accepted theory that the addition to the soil of readily decomposable substances of wide nitrogen-carbon ratio does cause a decrease in nitrates present; also in order to establish the fact that injuries to crops grown following the application of fresh manure are due to nitrate depletion rather than to the presence in the manure of toxic compounds or harmful bacteria.

TABLE 2

Decrease in soil nitrates following applications of fresh manure

TREATMENT	UNLIMED			LIMED		
	Nitrate nitrogen	Average	Variation from check	Nitrate nitrogen	Average	Variation from check
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Check	2.00 1.67	1.84	......	3.16 3.16	3.16	......
0.25 gm. manure	2.02 2.25	2.14	+0.30	3.55 3.34	3.45	+0.29
0.5 gm. manure	1.62 1.47	1.55	−0.29	4.33 2.70	3.52	+0.36
1.0 gm. manure	2.11 1.78	1.95	+0.11	1.35 1.54	1.45	−1.71
1.5 gm. manure	2.11 1.78	1.95	+0.11	0.59 0.77	0.68	−2.48
2.0 gm. manure	0.10 0.19	0.15	−1.69	Trace Trace	Trace	−3.16

The experiment was carried out with a Norfolk sand, deficient in nitrogen, low in organic matter content, and having a lime requirement of 700 pounds of calcium carbonate to the acre foot. One-hundred-gram portions of the air-dried soil were weighed out into tumblers and dry, sterilized manure having a nitrogen content of 1.18 per cent was added at the rates shown in table 2. Half of the tumblers received 1 gm. each of finely ground limestone. Water was added to bring the moisture content up to 45 per cent of saturation, allowing 2 cc. additional for each gram of manure used. The mixtures were allowed to incubate at room temperature for 25 days, and then the nitrates were leached out and determined by the phenol-disulfonic acid method. The results are given in table 2.

The data of table 2 show that the supply of soil nitrates was greatly reduced following applications of manure. In the absence of lime, small quantities had little effect, although 2 per cent reduced the supply approximately 92 per cent within 25 days. In the presence of calcium carbonate the nitrate supply was slightly increased by a maximum of 0.5 per cent manure, but with larger quantities the nitrates decreased to even less than with no lime. With 2 per cent of manure, only a trace of nitrates remained. These data seem to show that whereas ground limestone is favorable to the nitrifying organisms, it also stimulates the other microörganisms, especially the ammonifiers, which produce no nitrates but, on the contrary, use them for their own growth. The manure and lime thus worked together to the same end, the manure furnishing the energy for an increased number of bacteria and fungi while the calcium carbonate helped to improve the environment by neutralizing the soil acids.

TABLE 3

Effect of fresh manure on the ammonia content of the soil

TREATMENT	AMMONIA NITROGEN	AVERAGE	VARIATION FROM CHECK	AMMONIA NITROGEN	AVERAGE	VARIATION FROM CHECK
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Check	3.53 2.02	2.78	......	1.18 2.13	1.66	......
0.5 gm. manure	1.12 1.06	1.09	−1.69	1.26 1.18	1.22	−0.44
1.0 gm. manure	2.80 2.18	2.49	−0.29	1.62 2.80	2.21	+0.55
3.0 gm. manure	1.18 1.46	1.32	−1.46	1.34 1.29	1.32	−0.34
5.0 gm. manure	1.29 0.84	1.07	−1.71	2.18 1.62	1.90	+0.24
10.0 gm. manure	2.44 2.13	2.29	−0.49	2.07 3.36	2.72	+1.06

Ammonification of fresh manure in soil

When the above nitrification experiment was started, a second series of tumblers was arranged for ammonia determinations. A fertile Sassafras sandy loam having a slight acidity was used in the moist condition. Fresh manure was added at rates varying from 0 to 10 gm. per 100 gm. of soil. Half of the tumblers received 1 gm. of calcium carbonate each as in the nitrification experiments. After mixing, 2 cc. of water was added for each gram of manure used. The inoculation period was 7 days, after which ammonia was determined by heat distillation with magnesium oxide.

The results , given in table 3, are somewhat erratic and frequently duplicate determinations vary to a considerable extent. This is largely due to the inaccuracy of the method for determining ammonia, especially in small amounts, in soils. However, in the absence of lime, the manure reduced the ammonia content of the soil. Lime alone reduced the ammonia, but when used with the manure had little effect. Since the incubation period was much shorter and a different soil used, these results could not be expected to bear any correlation to the nitrification experiment already reported.

Rate of nitrification of fresh manure in soil

After having determined that the quantity of nitrates in the soil is decreased by the addition of fresh manure, it seemed desirable to learn whether the quantity of nitrates later returns to normal and, if so, how long a period is required for this to take place in the presence of different quantities of the manure. A series of experiments was, therefore, started using a fresh Sassafras sandy loam soil which was slightly acid and fairly productive. To all tumblers was added 1 gm. of calcium carbonate. The manure was thoroughly mixed with the soil and 2 cc. of water added for each gram as in previous experiments. The treatments and period of incubation together with the final results are given in table 4. To two tumblers was added ammonium sulfate without manure in order to test the nitrifying power of the soil.

The depression in nitrates following the application of fresh manure took place immediately after the addition and was greatest with the largest amount applied. With a 3 per cent treatment, only a trace of nitrates remained after 16 days. However, it is significant that after 40 days there was a partial recovery from the nitrate deficiency and at the end of the experiment (66 days) more nitrates were present in the treated than in the untreated soils.

These results are very significant from a practical standpoint and show that fresh manure of the type used here should not be applied just before or after seeding to crops which require an abundance of nitrate nitrogen. Furthermore, the data show that if the fresh manure is allowed a period of about six weeks under conditions which favor rapid biological action, the carbohydrates will be sufficiently used up to permit of a rapid nitrate formation and accumulation if not used by plants. This condition exists in a compost heap and table 4 merely emphasizes the desirability in many instances of composting and shows why such a procedure gives good results. Where composting is not practical, the undecomposed manure should be applied at such a time and place as to interfere least with the crop rotation. Where a crop is to be grown immediately following the application, a so-called "rank feeder" crop, such as corn, should be grown. This crop uses readily forms of nitrogen other than nitrates, especially ammonia, and is therefore less affected by a nitrate deficiency.

TABLE 4

Rate of nitrification of fresh manure in fresh soil

TREATMENT	7 DAYS			16 DAYS			25 DAYS			40 DAYS			66 DAYS		
	Nitrate N	Average	Variation from check	Nitrate N	Average	Variation from check	Nitrate N	Average	Variation from check	Nitrate N	Average	Variation from check	Nitrate N	Average	Variation from check
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Check	1.56 1.78	1.67	......	2.07 1.91	1.99	......	2.07 2.07	2.07	.....	2.25 2.45	2.35	....	1.52 1.57	1.54	....
0.25 gm. manure	1.91 1.78	1.84	+0.17	1.25 1.56	1.40	−0.59	1.19 0.98	1.08	−0.99	2.25 1.37	1.81	−0.54	1.52 1.62	1.57	+0.03
0.5 gm. manure	1.56 1.25	1.40	−0.27	0.84 0.70	0.77	−1.22	0.41 0.49	0.45	−1.62	1.25 1.41	1.33	−1.02	1.68 1.90	1.79	+0.25
1.0 gm. manure	0.98 1.40	1.19	−0.48	0.39 0.46	0.42	−1.57	0.31 0.28	0.29	−1.78	0.71 0.91	0.81	−1.54	2.18 2.78	2.48	+0.94
2.0 gm. manure	0.91 0.64	0.77	−0.90	0.36 0.39	0.37	−1.62	0.14 0.15	0.14	−1.93	0.53 0.41	0.47	−1.88	Lost 2.91	2.91	+1.37
3.0 gm. manure	0.42 0.56	0.49	−1.18	Trace Trace	Trace	−1.99	Trace Trace	Trace	−2.07	Lost 0.35	0.35	−2.00	4.48 5.18	4.83	+3.29
100 mgm. ammonium sulfate	....	....	....	....	.....	....	13.33 12.22	12.77	+10.70	....	....	....	....	....	....

Recovery of nitrates from soils fertilized with sodium nitrate and fresh manure

With the results given in table 4 in mind, it seemed desirable to carry the idea further by determining the rate at which sodium nitrate disappears from soils receiving fresh stable manure. By using the same methods as in previous nitrification experiments, including the addition of calcium carbonate, the results given in table 5 were obtained. A rather rich Penn loam soil was chosen; in fact, as was later discovered, too well supplied with nitrate nitrogen to be best suited for the experiment planned.

The results secured were in close agreement with the work previousy reported. The application of fresh manure hastened the removal of the nitrates that were present in the soil or were added to it in the form of sodium nitrate, and the larger the application, the greater the depression. Usually the smallest quantity of nitrates was present at the end of 21 days, after which there was a recovery in some instances, but usually the nitrate supply remained near the low point until the end of the experiment (83 days). It is rather surprising that such a large proportion of the added nitrate utilized by the microörganisms soon after its addition failed to nitrify again later and accumulate as such.

Nitrification of ammonium sulfate in the presence of fresh manure

The rate of nitrification of ammonium sulfate in the presence of fresh manure was determined on the same soil as in the preceding experiment and by the same methods of experimentation. The application of 100 mgm. of ammonium sulfate per 100 gm. of soil was made in solution. Table 6 gives the results in detail.

The data show that in the absence of manure the ammonium sulfate nitrified very readily, the largest nitrate accumulation being at the end of 21 days. With 1 per cent of manure, the nitrification was considerably slower, reaching a maximum in 52 days, after which the nitrate supply was used up very rapidly. With 2 per cent of manure, the nitrification was slower and the maximum percentage of the ammonium sulfate nitrogen which was recovered as nitrates was only about half that for the other two treatments mentioned. It is, of course, impossible to state from these data whether in the presence of the manure the ammonium sulfate nitrified at a normal rate, the nitrates then being used by microörganisms, or whether the ammonium sulfate was used as such. Doubtless a considerable portion of the nitrogen was used as ammonia.

Effect of fresh manure on the growth of buckwheat fertilized with an excessive amount of sodium nitrate

Since the laboratory experiments had shown in such a positive manner that heavy applications of fresh manure result in a rapid depletion of the soil nitrates it was decided to follow up the findings by plant studies in the greenhouse. Pots holding 1600 gm. of air-dried sandy loam soil were fertilized with so-

TABLE 5

Effect of the addition of fresh manure on soil nitrates

TREATMENT	13 DAYS			21 DAYS			52 DAYS			83 DAYS		
	Nitrate N	Average	Decrease over check	Nitrate N	Average	Decrease over check	Nitrate N	Average	Decrease over check	Nitrate N	Average	Decrease over check
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated	12.75 11.17	11.96	.....	9.75 11.10	10.43	.....	9.00 10.80	9.90	.....	10.05 9.60	9.83	.....
1 gm. manure	9.94 8.63	9.29	2.67	5.85 5.70	5.78	4.65	4.50 4.95	4.73	5.17	6.75 6.75	6.75	3.08
2 gm. manure	4.13 3.19	3.66	8.30	2.10 1.65	1.88	8.55	2.40 3.45	2.93	6.97	6.00 6.15	6.08	3.75
5 mgm. N as $NaNO_3$	16.02 16.88	16.45	.....	12.86 14.36	13.61	.....	15.00 15.64	15.32	.....	16.08 15.00	15.54	.....
5 mgm. N as $NaNO_3$ + 1 gm. manure	11.82 10.12	10.97	5.48	9.64 10.92	10.28	3.33	6.75 6.00	6.38	8.94	11.55 10.80	11.18	4.36
5 mgm. N as $NaNO_3$ + 2 gm. manure	8.63 9.19	8.91	7.54	5.36 6.00	5.68	7.93	7.50 7.80	7.65	7.67	6.75 7.05	6.90	8.64
10 mgm. N as $NaNO_3$	19.00 19.52	19.26	.....	16.75 16.25	16.50	.....	20.00 18.25	19.13	.....	25.20 22.50	23.85	.....
10 mgm. N as $NaNO_3$ + 1 gm. manure	13.87 12.19	13.03	6.23	11.78 12.43	12.10	4.40	12.86 12.86	12.86	6.27	12.45 11.58	12.02	11.83
10 mg. N as $NaNO_3$ + 2 gm. manure	12.38 12.00	12.19	7.07	9.64 9.00	9.32	7.18	9.64 10.71	10.18	8.95	9.64 9.86	9.75	14.10

TABLE 6

Nitrification of ammonium sulfate in the presence of fresh manure

TREATMENT	13 DAYS			21 DAYS			52 DAYS			83 DAYS		
	Nitrate N	Average	Increase over check	Nitrate N	Average	Increase over check	Nitrate N	Average	Increase over check	Nitrate N	Average	Increase over check
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated	12.75 11.17	11.96	.....	9.75 11.10	10.43	.....	9.00 10.80	9.90	.....	10.05 9.60	9.83	.....
Ammonium sulfate	25.00 23.75	24.38	12.42	26.66 24.00	25.33	14.90	22.50 21.00	21.75	11.85	25.20 22.50	23.85	14.02
Ammonium sulfate + 1 gm. manure	20.62 15.93	18.28	6.32	23.33 21.67	22.50	12.07	25.00 24.67	24.84	14.94	Lost 16.67	16.67	6.84
Ammonium sulfate + 2 gm. manure	18.75 19.00	18.88	6.92	13.67 15.00	14.34	3.91	16.00 18.33	17.17	7.27	14.66 15.00	14.83	5.00

dium nitrate and dry manure as shown in table 7. Sodium nitrate was applied in amounts large enough to be injurious when used alone. The crop grown was buckwheat, which was harvested when about 18 inches high and in full bloom. The weight of dry matter is given in table 7.

As shown by the figures, the large application of sodium nitrate alone or with the smallest application of manure resulted in a very poor growth—about 13 per cent of the untreated check. With the 5- and 10-gm. applications of manure, the dry weight increased 70 per cent over the check. The laboratory experiments indicate that the manure caused such a rapid multiplication of microörganisms with the consequent utilization of the nitrates that the normal growth of the buckwheat could take place.

This experiment suggests a possible method of preventing the loss of nitrates from soils during the summer or fall, when nitrification is taking place. Instead of planting a cover crop, the same object might possibly be obtained by plowing under a heavy application of fresh stable manure, straw, weeds or other materials having a high ratio of carbon to nitrogen. This would be practicable only to a limited extent and under special conditions.

TABLE 7

Effect of fresh manure on the growth of buckwheat fertilized with an excessive amount of sodium nitrate

TREATMENT	WEIGHT DRY MATTER	AVERAGE	VARIATION FROM CHECK
	gm.	*gm.*	*per cent*
Untreated	1.7 1.3	1.50	......
0.53 gm. $NaNO_3$	0.2 0.2	0.20	−86.7
0.53 gm. $NaNO_3$ + 2.5 gm. manure	0.2 0.2	0.20	−86.7
0.53 gm. $NaNO_3$ + 5 gm. manure	2.4 2.7	2.55	+70.0
0.53 gm. $NaNO_3$ + 10 gm. manure	2.4 2.7	2.55	+70.0

Effect of fresh manure on the growth of wheat in the greenhouse

Another series of pot experiments on the same soil and with the same methods, was started to determine the effect of stable manure, both in the moist condition and dry and sterile, on the growth of wheat. The manure was used alone and also with sodium nitrate. Table 8 gives the green weights of wheat from one series of pots in which growth was allowed to continue for 33 days and from a second group of pots in which the wheat grew to maturity. Plate 1 shows the appearance of the wheat at the end of 24 days.

As a whole, these data obtained in the greenhouse agree very closely with what might be expected from the laboratory results already discussed. The fresh manure, whether applied in the moist unsterilized or dry and sterile condition was noticeably injurious to the growth of wheat. Where sodium nitrate was also added, the growth and yields were usually larger than with the nitrate alone, showing definitely that the injury caused by the manure was merely due to a nitrate starvation and not to any toxic constituents in the manure or to any harmful organisms added. In fact, the sterile manure was slightly more harmful than the moist fresh manure and acted exactly the same as would an application of straw, sugar, or other readily decomposable materials having a wide nitrogen-carbon ratio. It is significant to note, also, that the wheat plants never recovered from the early nitrate starvation and the decrease in grain yields was somewhat comparable to the early growth effects.

TABLE 8

Effect of fresh manure on the growth of wheat in the greenhouse

TREATMENT	GREEN WEIGHTS AFTER 33 DAYS		DRY WEIGHTS AT MATURITY			
	Total weights	Variations from check	Weight of straw	Weight of grain	Total weight	Variations from check
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
No fertilizer	7.4	......	2.9	1.1	4.0	......
0.5 gm. $NaNO_3$	15.4	+8.0	6.5	2.5	9.0	+5.0
5 gm. dry manure	6.0	−1.4	1.9	0.6	2.5	−1.5
5 gm. dry manure + 0.5 gm. $NaNO_3$	18.8	+11.4	6.6	2.3	8.9	+4.9
10 gm. dry manure	4.0	−3.4	1.7	0.7	2.4	−1.6
10 gm. dry manure + 0.5 gm. $NaNO_3$	17.8	+10.4	6.1	2.1	8.2	+4.2
16 gm. wet manure	6.5	−0.9	2.5	1.0	3.5	−0.5
16 gm. wet manure + 0.5 gm. $NaNO_3$	19.6	+12.2	7.4	2.6	10.0	+6.0
32 gm. wet manure	5.8	−1.6	2.5	1.0	3.5	−0.5
32 gm. wet manure + 0.5 gm. $NaNO_3$	19.8	+12.4	7.2	2.5	9.7	+5.7

DISCUSSION

The data as presented in this paper emphasize forcibly the importance of giving consideration to the nitrogen-carbon ration and to the availability of the energy and nitrogen present in organic materials added to soils as fertilizers. A close relation exists between the carbon and the nitogen content of soils, the ratio of nitrogen to carbon being about 1 to 10 in most cultivated soils and somewhat wider in virgin soils. Virgin soils which have accumulated an excess of organic matter over a period of years support a varied and active microflora on being put under cultivation. These organisms consume a considerable portion of the organic matter, particularly the carbon. A narrowing of the nitrogen-carbon ratio to near the usual 1:10 constant results. Exactly the same thing happens when the ordinary cultivated soil receives a heavy application of organic fertilizer. If the added material, such as dried blood, has a narrow nitrogen-carbon ratio, then it is attacked by the ammonifying organisms and free ammonia is produced. A portion of this ammonia may be lost, depending upon the conditions. At least there is a tendency for this to occur and for the nitrogen-carbon ratio to be restored to normal. On the other hand, as is proved in this paper, if materials having a wide nitrogen-carbon ratio, such as fresh stable manure, straw, sugar, and most woody plant residues, are added to the soil, the excess energy is attacked by the soil organisms. The result is a vigorous evolution of carbon dioxide and a narrowing of the nitrogen-carbon ration to near normal. These vigorously growing organisms require nitrogen, particularly nitrates, for their activities and hence remove most or all that is available from the soil solution. Any crop growing on the soil at the time must necessarily suffer nitrate starvation or a "burning" of the foliage. Later, after the bacterial activities subside the nitrogen existing as protein in the bacterial cells is ammonified and the ammonia in turn oxidized to nitrates. The immediate result of the addition of materials of a wide nitrogen-carbon ratio is often harmful, but the ultimate effect is beneficial, provided sufficient time is allowed for the nitrate supply to return to normal.

The procedure to be followed in applying organic materials to soils, particularly those materials which decompose readily and have wide nitrogen-carbon ratios, must depend upon the particular conditions existing. Several alternatives present themselves; the materials may first be allowed to decay partially, preferably in a compost heap; they may be applied several weeks before planting and be allowed to decay in the soil; and in certain exceptional conditions they may be applied immediately before planting in limited quantity and provided that the crop to be grown can use forms of nitrogen other than nitrates. As previously stated, corn is thought to be a crop of this nature. The amount of information available on this subject is, however, limited.

Another point brought out by the data here presented is the fact that the well-known toxic properties of fresh stable manure cannot be attributed to any particular harmful factor present, either of a chemical or biological nature, for straw or sugar produces the same type of injuries. Fresh manure, therefore, is merely another example of a material having a wide nitrogen-carbon ratio and being easily decomposed by soil microörganisms. Any soil treatment which hastens the oxidation of the carbon will intensify, at least for a short time, the nitrate starvation.

SUMMARY

This paper presents the results of laboratory and greenhouse studies on nitrate assimilation by soil microörgaisms, the results of which may be summarized as follows:

1. The addition to soils of readily decomposable materials of wide nitrogen-carbon ratio results in a marked increase in biological activities and a decrease in soil nitrates due to their assimilation by the soil microörganisms. This utilization of the nitrates causes an injury to most growing crops, almost wholly because of nitrate starvation. The addition of sodium nitrate under such conditions caused the plants to grow normally or even better than on the untreated soils.
2. The injury caused by fresh stable manure is not due to any particular toxic compound present or to the microörganisms added, but is due to the stimulation of biological activities with the resulting nitrate assimilation. There is no appreciable difference in the nature of the injury produced by such materials as fresh stable manure, timothy hay, straw, and corn stover. Any difference noted is largely one of degree because such materials contain varying percentages of nitrogen and carbon and decompose with varying degrees of rapidity.
3. The injury caused by the addition of such materials as those mentioned is not permanent but continues until there is a slowing up of biological activities and until there is sufficient time for the protein in the dead bacterial cells to be made available for plants through ammonification and nitrification. In these experiments, where conditions were optimum for rapid bacterial action, the period lasted from 4 to 8 weeks but of course this period would vary widely under field conditions, depending upon the rate of application and soil conditions.

REFERENCE

(1) Brown, P. E., and Allison, F. E. 1916 Influence of humus forming materials of different nitrogen-carbon ratios on bacterial activities. *Iowa Agr. Exp. Sta. Res. Bul.* 36: 25–28.

PLATE 1

EFFECT OF FRESH MANURE ON THE GROWTH OF WHEAT. PHOTOGRAPHS TAKEN 24 DAYS AFTER PLANTING

FIG. 1. *a*, no fertilizer; *b*, 0.5 gm. $NaNO_3$; *c*, 5 gm. dry manure; *d*, 5 gm. dry manure and 0.5 gm. $NaNO_3$; *e*, 10 gm. dry manure.

FIG. 2. *a*, 10 gm. dry manure and 0.5 gm. $NaNO_3$; *b*, 16 gm. wet manure; *c*, 16 gm. wet manure and 0.5 gm. $NaNO_3$; *d*, 32 gm. wet manure; *e*, 32 gm. wet manure and 0.5 gm. $NaNO_3$.

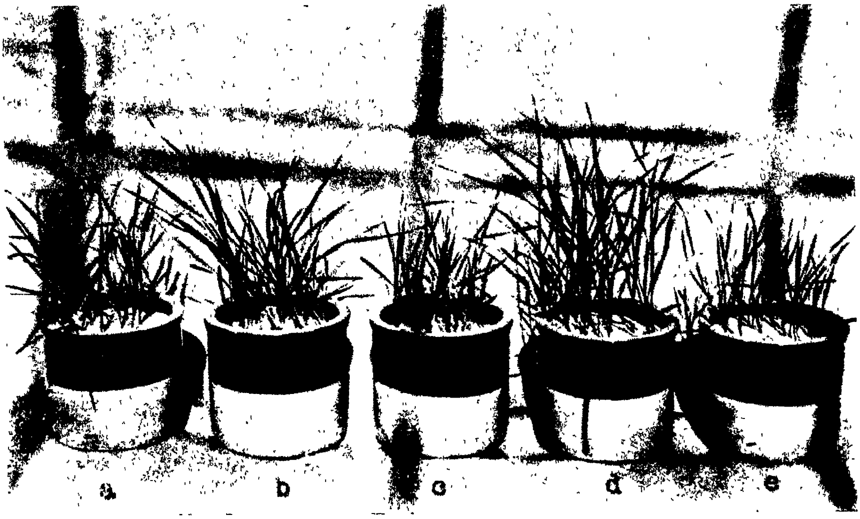

FIG. 1

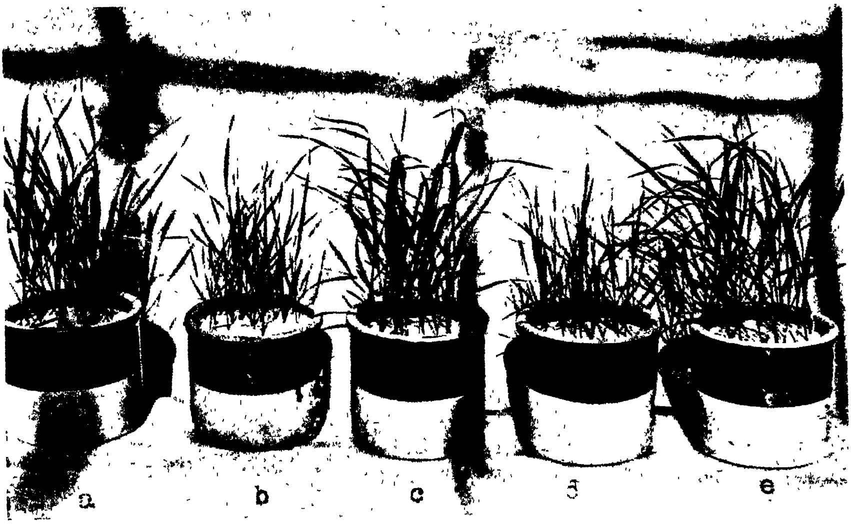

FIG. 2

THE INFLUENCE OF MANURES AND ORGANIC RESIDUES ON PLANT GROWTH[1]

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INTRODUCTION

Under conditions of intensive farming where large amounts of plant-food need to be available at specific times there is much objection to the use of manures containing appreciable amounts of shavings, sawdust, or straw, because of their adverse influence on the immediate crop. This influence is commonly attributed to some toxic material which is supposed to be either present in these organic substances when they are applied or developed when these substances undergo decomposition. In the case of sawdust and shavings the depressing influence on yield has been attributed to the presence of turpentine.

New lands from which a stand of pine timber has been removed as a rule produce comparatively poor crops immediately after clearing, a condition that is gradually overcome in the course of a few years of cropping. These poor crops are generally attributed to the presence of turpentine, or at least to a toxic effect of the pine needles themselves or their residue when plowed under (3).

Under conditions of grain farming, especially in the drier regions, where summer fallowing is the common practice, the maintenance of soil organic matter has become a vital problem. When straw is returned, in attempts to maintain the organic matter, reduced yields are so frequently experienced that the practice has little to encourage it. These reduced yields until recently have been generally attributed either to the dryness of soil resulting from the dry straw or to some toxic effect of the straw itself or to both. It is now generally recognized, however, that during the decomposition of organic materials low in nitrogen, reactions occur in the soil that furnish a very satisfactory explanation for the resulting decreased yield.

Results of soil nitrate determinations made by numerous investigators, for instance, show conclusively that the application to the soil of straw, sawdust, shavings, or any organic material low in nitrogen content has a tendency to depress nitrate accumulation, with the result that soils receiving an application of such low nitrogen materials contain less nitrate nitrogen after the application than before. Some investigators, therefore, have attributed the low yields both to the reduction of nitrates and also to the toxic effect of some of the products of decomposition (1).

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[1] Originally submitted partially to satisfy the requirements for a master's degree in Plant Pathology at the State College of Washington. Acknowledgment is accorded to Professors F. J. Sievers and H. F. Holtz of the Soils Department of the College for valuable criticisms and suggestions.

TABLE 1

Yield and nitrogen content of oats grown on Palouse silt loam under various treatments with low nitrogen materials, and with nitrate of soda

TREATMENT OF SOIL	YIELD STRAW AND GRAIN	YIELD GRAIN	NITROGEN GRAIN	NITROGEN GRAIN
	gm.	*gm.*	*per cent*	*gm.*
Control	9.2	3.38	1.75	0.0592
Straw, 2 T.	3.6	1.25	1.76	0.0220
Straw, 2 T.; $NaNO_3$, 150 pounds	5.3	1.97	1.92	0.0379
Straw, 2 T.; $NaNO_3$, 600 pounds	19.4	6.74	1.71	0.1153
Pine needles, 2T.	6.8	2.60	1.71	0.0445
Pine needles, 2 T.; $NaNO_3$, 150 pounds	10.6	3.65	1.81	0.0660
Saw dust, 2 T.	6.3	2.18	1.93	0.0421
Saw dust, 2 T.; $NaNO_3$, 150 pounds	10.8	3.95	1.51	0.0596

TABLE 2

Yield and nitrogen content of oats grown on Palouse silt loam under various treatments with manures, and with nitrate of soda

TREATMENT OF SOIL	YIELD STRAW AND GRAIN	YIELD GRAIN	NITROGEN GRAIN	NITROGEN GRAIN
	gm.	*gm.*	*per cent*	*gm.*
Control	9.2	3.38	1.75	0.0592
Fresh manure, 5 T.	12.6	4.69	1.82	0.0854
Fresh manure, 5 T.; $NaNO_3$, 150 pounds	13.6	5.20	1.64	0.0853
Rotted manure, 5 T.	9.4	4.03	1.70	0.0686
Rotted manure, 5 T.; $NaNO_3$, 150 pounds	12.0	5.07	1.66	0.0842
$NaNO_3$, 150 pounds	11.5	4.15	1.77	0.0724
$NaNO_3$, 300 pounds	13.5	5.14	1.63	0.0838
$NaNO_3$, 600 pounds	19.3	7.18	1.66	0.1192

PLAN OF THE EXPERIMENT

In the following experiment three materials of low nitrogen content; namely, sawdust, pine needles, and straw, were used for soil treatment in an endeavor to determine their direct influence on the growth and yield of oats and also to determine whether their depressing effect, if any, could be overcome by treatment with a nitrogen fertilizer. Also fresh and rotted manures are compared both when applied alone and when supplemented with a nitrogen fertilizer. The organic materials of low nitrogen content were added at the rate of 2 tons, and the manures at the rate of 5 tons to the acre.

The soil used was a Palouse silt loam containing about 0.18 per cent nitrogen to the acre 7 inches deep. The soil was thoroughly pulverized, and after the different treatments it was put into gallon cans and immediately planted to oats.

DISCUSSION OF DATA

For convenience in considering the data the tables are divided into two groups conforming with the two illustrations. Table 1 and figure 1 form the first group, and table 2 and figure 2 the second.

In group 1, which contains all the residues of low nitrogen content, the effect of the addition of straw alone to the soil becomes very evident when the first two pots both in figure 1 and table 1 are compared.

The influence of adding nitrogen to soil to which a residue of low nitrogen content has been added is well brought out by a comparison of the three pots in figure 1 containing the same amount of straw. Of these the two in which the straw was supplemented by nitrogen show an increase in tillering, growth, and yield. This increase was in nearly direct proportion to the amount of nitrogen added.

The same influence of the nitrogen supplement to residues of low nitrogen content is evident where pine needles and sawdust were used as residues instead of straw. There is this difference, however, that the depressing effect of straw was the most pronounced, although all lowered the yield considerably below that of the control.

In group 2 the contrasts resulting from different treatments are not so great. This is as should be expected since none of these pots contain materials of such low nitrogen content as do the pots of group 1. In group 2 the control shows the poorest growth, the least tillering, and the lowest yield. Here also, as in group 1, wherever nitrogen was added with the residues the yield was correspondingly increased.

In this connection it should be borne in mind that all pots were kept supplied with water to satisfy their requirements. Irrespective of the amount of tillering due to variations in available nitrogen in the soil, enough water was supplied to bring all plants to a normal maturity. Even under field conditions, where such an ideal moisture relation would seldom exist, the same general results from the addition of low nitrogen residues would obtain (4).

The pots to which the greatest amount of nitrogen was added produced the highest yield and removed the most nitrogen in the grain, but did not necessarily produce grain having the highest percentage of this element. This low percentage of nitrogen may be explained on the basis that where the nitrogen supplement is added at the time of planting, as in this experiment, it tends to be utilized in promoting increased tillering, growth, and yield, rather than in increased percentage of nitrogen in the grain as would be the case were the nitrogen application made after the tillering stage (2).

On the other hand the pots yielding grain with the highest percentage of nitrogen were the ones treated with 2 tons of straw plus 150 pounds of nitrate of soda, and with 2 tons of sawdust alone to the acre. This seems to show that although the residues of low nitrogen content retarded tillering, growth, and yield, their depressing effect on nitrate accumulation was sufficiently overcome during the later stages of the plants' development to permit this extra nitrogen to become available. The nitrogen so made available at this late period of maturity was used to increase the percentage of nitrogen in the grain, for it was then too late to influence yield through further tillering.

In the case of the fresh manure, indications are that either the manure used was of such quality that it contained comparatively little material of low nitrogen content, or otherwise the control conditions of adequate moisture supply caused decomposition to proceed very rapidly with the result that there was a comparatively liberal supply of nitrate nitrogen. The result is that although the total yield of the pot with the fresh manure plus the nitrogen supplement is more than where fresh manure is used alone, the total nitrogen removed by the two pots is practically the same. The depressing effect of any residues of low nitrogen content contained in this fresh manure apparently was not enough to prevent the nitrogen of the manure from raising the yield well above that of the control.

In the case of the rotted manure under these conditions of optimum moisture supply, the nitrogen became available less rapidly than with fresh manure and there was a corresponding influence on yield.

The results from the foregoing experiment are consistent with those contained in other literature bearing on the subject, in that they seem to point to the conclusion that in the decomposition of organic matter the one important factor is the carbon-nitrogen ratio of the material undergoing decomposition.

CONCLUSION

The conclusion reached from the data presented is that the depressing effect on growth and yield immediately following the addition of low nitrogen materials to soil may be largely overcome by applying sufficient quantities of nitrate of soda as a supplement.

REFERENCES

(1) Collison, R. C., and Conn, H. J. 1925 The effect of straw on plant growth. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 114.

(2) Davidson, J., and LeClerc, J. A. 1923 Effect of various inorganic nitrogen compounds, applied at different stages of growth, on the yield, composition, and quality of wheat. *Jour. Agr. Res.* 23: 55–68.

(3) Gibbs, W. M., and Werkman, C. H. 1922 Effect of tree products on bacteriological activity in soil: I. Ammonification and nitrification. *Soil Sci.* 13: 303–322.

(4) Sievers, F. J., and Holtz, H. F. 1926 The significance of nitrogen in soil organic matter relationships. Wash. Agr. Exp. Sta. Bul. 206.

PLATE 1

EFFECT OF ORGANIC RESIDUES ON GROWTH AND YIELD OF OATS

FIG. 1. Effect of residues of low nitrogen content. *C*, control. *S*, straw, 2 tons to the acre *PN*, pine needles, 2 tons to the acre. *SD*, sawdust, 2 tons to the acre. *N*, nitrate of soda The numerals indicate the number of pounds of nitrate of soda applied to the acre.

FIG. 2. Effect of residues of higher nitrogen content. *C*, control. *FM*, fresh manure 5 tons to the acre. *RM*, rotted manure, 5 tons to the acre. *N*, nitrate of soda. The numerals indicate the number of pounds of nitrate of soda applied to the acre.

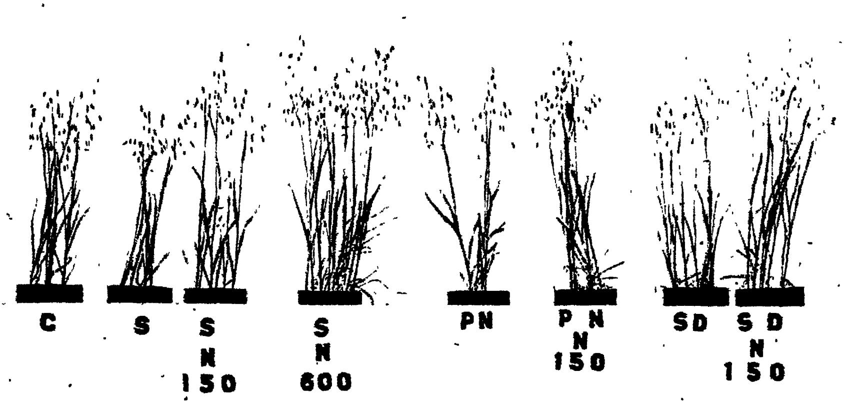
C
S
S N 150
S N 600
PN
P N N 150
SD
S D N 150

Fig. 1

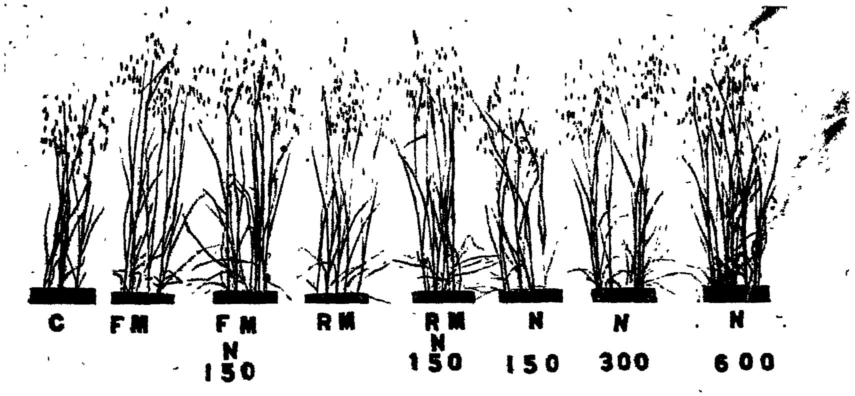
C
FM
F M N 150
RM
RM N 150
N 150
N 300
N 600

Fig. 2

PRODUCTION OF NODULES ON DIFFERENT PARTS OF THE ROOT SYSTEMS OF ALFALFA PLANTS GROWING IN SOILS OF DIFFERENT REACTION

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Most legume plants grow best in neutral or nearly neutral soils, in part at least because of the favorable conditions for activities of the nodule bacteria in such soils. Information was desired as to what extent soil reaction directly affects nodule production and presumably the activity of the nodule bacteria and to what extent indirectly through some general effect on the host plant. Truog has expressed the belief that the effect of soil reaction on the activity of the bacteria in the nodule is indirect since the environment of the bacteria when in the nodule must be that of the plant tissues (3). This seems a rather obvious conclusion, but it does not preclude the possibility that the effect on the plant tissues is local in nature, or perhaps greater on the nodule tissues than on the plant generally. Soil reaction may also affect nodule development through its effect on the bacteria when existing non-symbiotically in the soil in the absence of the host plant.

To secure the desired information, experiments were conducted on legume plants arranged so that one part of the root system of any one plant was growing in acid soil and the other part in limed soil. A smaller production of nodules on the roots grown in the acid than in the limed soil, particularly if the difference was approximately as great as that between root systems grown entirely in acid and limed soils, seemingly would show a direct effect of soil reaction on nodule development. On the other hand, if the effect is wholly or largely an indirect one, arising from some general effect on the plant, not only should the difference in production of nodules between the two parts of any root system thus grown be much less than between entire root systems grown respectively in acid and limed soils, but the effect of the acid soil should be to depress production of nodules on that part of the root system growing in the limed soil as compared with production of nodules on the corresponding part of root systems grown entirely in limed soil.

Alfalfa was selected because of its sensitiveness to soil reaction. The plants were grown in containers mainly in the greenhouse. Soil from limed and unlimed plots of certain Kentucky fertility experiment fields was used. The differences in soil reaction were thus those brought about by liming under field conditions. Paraffine-painted wooden boxes with inside dimensions 10 inches each way were used for containers in the first experiments. Paraffine-painted galvanized iron partitions, extending to within 1 inch of the soil surface, divided these boxes vertically into two equal parts. The seeds were planted in a row directly above the partition. A difficulty with these experiments was the uneven distribution of roots usually secured between the two parts, the larger part of the roots developing in the limed side. The same difficulty was experienced in later experiments in which the vertical division but no actual partition was used.

TABLE 1

Plan of the experiment, weight of plants, and number of nodules with the Mayfield soil in the first experiment

POT NUMBER	SURFACE SOIL	UNDER SOIL	AIR-DRY WEIGHT OF TOPS	AIR-DRY WEIGHT OF ROOTS			NUMBER OF NODULES		
				Surface soil	Under soil	Total	Surface soil	Under soil	Total
			gm.	*gm.*	*gm.*	*gm.*			
1	1 inch acid	5 inches limed	1.991	0.170	0.263	0.433	0	102	102
2	2 inches acid	4 inches limed	1.013	0.096	0.165	0.261	0	20	20
3	3 inches acid	3 inches limed	0.445	0.103	0.062	0.165	0	30	30
4	1 inch limed	5 inches acid	1.190	0.115	0.129	0.244	27	1	28
5	2 inches limed	4 inches acid	0.988	0.148	0.065	0.213	41	3	44
6	3 inches limed	3 inches acid	2.320	0.345	0.175	0.520	94	15	109
7	Entire soil acid		0.403			0.139			8
8	Entire soil limed		2.260			0.796			104

TABLE 2

Plan of the experiment, weight of plants, and number of nodules with the Berea soil in the first experiment

POT NUMBER	SURFACE SOIL	UNDER SOIL	AIR-DRY WEIGHT OF TOPS	AIR-DRY WEIGHT OF ROOTS			NUMBER OF NODULES		
				Surface soil	Under soil	Total	Surface soil	Under soil	Total
			gm.	*gm.*	*gm.*	*gm.*			
11	1 inch acid	5 inches limed	2.540	0.270	0.664	0.934	0	20	20
12	2 inches acid	4 inches limed	0.739	0.081	0.075	0.156	0	15	15
13	3 inches acid	3 inches limed	0.246	0.052	0.017	0.069	0	3	3
14	1 inch limed	5 inches acid	0.128	0.011	0.621	0.032	0	0	0
15	2 inches limed	4 inches acid	0.182	0.020	0.025	0.045	1	0	1
16	3 inches limed	3 inches acid	0.616	0.114	0.036	0.150	10	0	10
17	Entire soil acid		0.053			0.017			0
18	Entire soil limed		2.094			0.790			20

The most satisfactory results were secured in experiments where the divisions were horizontal and where there was no actual partition between the two soil parts. The acid soil was the surface soil in some pots and the under soil in other pots in these experiments. Six-inch porous earthenware pots were used as containers in two experiments. These pots made possible a total soil depth of 6 inches. In the first of these experiments soils from the Mayfield and Berea experiment fields were used.

Satisfactory growth of red clover is never secured on the unlimed Berea soil and but rarely on the untreated Mayfield soil. Both soils are silt loams. The Mayfield soil is of loessal origin. The Berea soil is weathered largely from Devonian shale.

The plots used and their fertilizer treatments were: Mayfield 304, manure and acid phosphate; 306, manure, limestone, and acid phosphate; Berea 305, manure and rock phosphate; 307, manure, limestone, and rock phosphate. The treatments have been approximately 6 tons of manure, 2 tons of pulverized limestone, and 800 pounds of acid phosphate or 1600 pounds of rock phosphate to the acre for each of two four-year rotations (2). The soils had been in storage in an air-dry condition for some time previous to use in this experiment.

Electrometric determinations of the reactions of these soils showed Mayfield plot 304, pH 4.80; plot 306, pH 6.98; Berea plot 305, pH 4.73; plot 307, pH 6.00.

The plan of the experiment, shown in tables 1 and 2, was the same with the soils from the two fields.

The soils were brought to 15 per cent moisture and inoculating material was added before potting. Approximately 2000 gm. of air-dry soil was placed into each pot. The inoculating material was a suspension of soil from a field of vigorous alfalfa; the part used was that decanted after the bulk of the soil had settled. The amount of suspension added contained 3 gm. of inoculating soil to the pot.

Commercial seed of common alfalfa was distributed over the surface of the pots on April 28. Five plants in each pot were allowed to grow. The water content of the soil was kept as nearly at the optimum as possible by frequent watering with distilled water, without weighing. The plants were harvested on July 1 and 2. In harvesting, the soil mass was removed intact from the pots. The soil in the divided pots was separated along the division plane with a sharpened spatula. The roots were removed from the soil as carefully as possible by crumbling the soil and sieving.

Results from the experiments are given in tables 1 and 2. As shown by the nodule counts, differences in production of nodules resulted when different parts of the root systems of the same plants grew in soils of different reaction. Inspection showed the differences here to be essentially the same as between entire root systems of plants grown respectively in the acid and in the limed soils.

There is the possibility, however, that the small nodule production on the roots in the acid soil part in the divided pots was due to the effect of the soil reaction on the bacteria while they were necessarily existing non-symbiotically in the soil. Experiments show that alfalfa nodule bacteria may be very much affected by acid soil reaction under such conditions (1). Soil suspension was used for inoculating to make this possibility less likely. It would seem also that the adjacent limed soil would have been a constant source of infection, particularly in view of the frequent watering.

In this connection, an experiment was made in which, in addition to an initial application, inoculating material was added at weekly intervals during the growth of alfalfa plants, and the development of nodules was compared with that where only the initial application of inoculating material was given. Six-inch porous earthenware pots filled with limed and unlimed soils from the Mayfield and Greenville fertility fields were used. The initial application of inoculating material—pure cultures—was mixed through the soil of the pots. The weekly additions were made to the surface of the pots directly before water was added. The experiment was begun on December 20 and the plants were removed for examination on April 1. Only a few nodules had been produced in the acid soils. Fair production had occurred in the limed soils. The pots with weekly additions of culture material showed slightly greater production of nodules in perhaps the surface inch of soil, as determined by careful inspection, than the pots receiving only the initial application of culture material. This effect, however, was as pronounced with the limed soils as with the acid soils.

It is believed that the differences in nodule production between the acid and limed parts of the divided pots were not due to the effect of reaction on the bacteria while existing symbiotically. However, a definite statement cannot be made in this connection.

The results of the experiment can be viewed in another way that obviates this difficulty, however. If the effect of soil reaction on activity of bacteria in the nodule is indirect, or largely so, its effect when a part of the roots of any plant is growing in acid soil should be evident in a lesser production of nodules on the part of the roots in the limed soil than on the corresponding part of the roots of a plant grown entirely in limed soil. On the whole, both the nodule counts and the inspection of the roots at the time of removal showed that such was not the case in these experiments. With the Mayfield soil (table 1) the roots from the 5 inches of limed under soil in pot 1 had practically the same number of nodules as the entire roots from pot 8 containing only limed soil. Also, the roots from the 3 inches of limed surface soil in pot 6 had 94 nodules as against 104 nodules on the roots from pot 8. Inspection of the roots at the time of removal in the case of this soil showed, when the limed soil was the surface soil, greater production of nodules than on the corresponding part of root systems of plants grown altogether in limed soil.

In the second experiment, in addition to the soils from the Mayfield and Berea fields, soil was used from limed and unlimed plots from the Greenville field (2). In general the plan of this experiment was the same as the preceding. Results from this experiment as well as from the larger containers already referred to were uniformly in agreement with the experiment reported above and therefore will not be given in detail.

The experiments reported in this paper indicate that nodule production correlates with soil reaction fully as well when a particular reaction is acting on only a part of the roots of any plant, as when acting on the entire root system. The effect of soil reaction on nodule production, therefore, is independent of any antecedent general effect on the host plant. The effect is direct on the bacteria in the nodule, or the effect of the soil reaction in changing the nature of the plant tissues and thus indirectly affecting the nodule bacteria is so localized as to affect only that part of the roots of plants directly in the soil of the reaction in question.

SUMMARY

Alfalfa plants were grown in soils from limed and unlimed plots of certain of the Kentucky fertility experiment fields. The plants were grown in pots containing all acid and all limed soils and in pots with acid soil in one part and limed soil in the other part. The most useful division was a horizontal one with no actual partition between the two parts. Differences in nodule development could thus be told when the acid and limed soils were (*a*) acting on entire root systems of plants and (*b*) acting on different parts of the root system of the same plant. Differences in nodule development were found to be as great in the case of (*b*) as of (*a*), indicating that the effect of soil reaction is independent of any antecedent general effect of soil reaction on the host plant. The possibility was noted that the poor development of nodules in the acid soil might have been due to the effect of the acid soil on the bacteria during the interval when they were existing non-symbiotically in the soil. It was pointed out, however, that if the effect of soil reaction on the bacteria is through some first general effect on the host plant, nodule development on the roots in the limed part of the divided pots should be less than on the corresponding part of the root systems of plants grown entirely in limed soils. Such was not found to be the case. Apparently the soil reaction directly affects the bacteria in the nodule; or the antecedent effect of the soil reaction on the host plant, which in turn influences nodule development, is so localized as to affect only that part of the roots directly in the soil of the reaction in question.

REFERENCES

(1) Bryan, O. C. 1923 Effect of acid soils on nodule forming bacteria. *Soil Sci.* 20: 37–40.

(2) Roberts, G., and Ewan, A. E. 1920 1. Report on soil experiment fields. *Ky. Agr. Exp. Sta. Bul.* 228: 80–120.

(3) Truog, E. 1918 Soil acidity: 1. Its relation to the growth of plants. *Soil Sci.* 5: 169–195.

SOIL PHOSPHORUS STUDIES: I. THE COLORIMETRIC DETERMINATION OF ORGANIC AND INORGANIC PHOSPHORUS IN SOIL EXTRACTS AND THE SOIL SOLUTION[1]

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In studying the water-soluble constituents of soils it is very desirable to have methods of analysis that are rapid, accurate, and suitable for the determination of small quantities of a given element. Such methods are almost essential for the study of water-soluble phosphorus since it is found in very low concentrations in the displaced solution and water extract of most soils. In the studies made in this laboratory, two colorimetric methods have been used for the determination of phosphorus. The one most extensively used is the coeruleo—molybdate method of Deniges (5) that has been used by Atkins (1, 2). The second method is that of Fiske and Subbarow (6), which with similar methods has been extensively used in blood analysis.

Both methods are based on the same chemical reaction and both possess many advantages over most of the methods commonly used for the determination of phosphorus in soil extracts. Some of the outstanding advantages of the methods are: 1. They make possible the determination of both the organic and inorganic phosphorus of the soil solution or extract. 2. The procedures are short and a number of determinations can be made in a very short time, especially when only the inorganic phosphorus is determined. 3. Very few substances interfere with the determination and none of these interfering substances ordinarily occur in soil extracts in sufficient quantity to influence the determination. 4. Very small amounts of phosphorus can be determined. By the method of Deniges, 0.001 mgm. PO_4 can be satisfactorily determined in a volume of 50 cc.

The methods are based upon the fact that a suitable reducing agent, when added to an ammonium molybdate solution of a certain acidity, will produce a blue color if the solution contains inorganic phosphate. The blue color is due to the partial reduction of some of the molybdenum in the complex phosphomolybdate and, within certain limits it is proportional to the phosphate concentration. Taylor and Miller (10) first suggested the possibility of using the reaction as a basis for the colorimetric determination of phosphorus. Subsequently, Wu (11) made a detailed study of the chemistry of the reaction but did not develop a quantitative method. Deniges (5), Bell and Doisy (3), Briggs (4), and Fiske and Subbarow (6) developed methods for the quantitative determination of phosphorus. Deniges apparently developed his method without knowledge of the work being done in this country. Several investigators have shown that the intensity of the blue color produced from a given phosphate concentration is dependent upon the reducing agent used.

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The methods of Deniges and Fiske and Subbarow differ in several respects, the most important being in the use of different reducing agents. Deniges used stannous chloride while Fiske and Subbarow use 1, 2, 4-aminonaphtholsulfonic acid. The methods also differ somewhat in the concentration of acid and molybdate used in the determination. Deniges' method is approximately five times as sensitive as that of Fiske and Subbarow and is therefore better adapted to the determination of phosphorus in soil solutions and extracts. The Fiske and Subbarow method, on the other hand, is very useful for determining phosphorus in solutions of higher concentrations than can be studied by the other method.

This paper will present some of the results obtained in a study of the two methods, together with the procedures recommended for the determination of organic and inorganic phosphorus.

THE COLORIMETRIC METHOD OF DENIGES

Atkins (1, 2) has used the colorimetric method of Deniges in a study of the phosphorus content of natural waters and soil extracts. It has been used in the phosphate studies made in this laboratory. The procedure first used in this laboratory has been given in another publication (8). As a result of subsequent studies the procedure has been slightly modified and has been used in this laboratory for the determination of organic and inorganic phosphorus in the displaced solution and in soil extracts. The procedure recommended for the determination of the two forms of phosphorus follows:

Solutions needed

Reagent A—Mix 100 cc. of 10 per cent ammonium molybdate solution with 300 cc. of a 50 per cent (by volume) solution of H_2SO_4. The acid must be arsenic-free and the reagent should be kept in the dark or in a brown bottle.

Reagent B—To 0.5 gm. of powdered tin add 5 drops of a 4.0 per cent solution of $CuSO_4$ and 10 cc. of conc. HCl, arsenic-free. Warm to hasten the reaction and when the tin is in solution dilute to 50 cc. This reagent must be prepared fresh each day.

Standard PO_4 solution—Dissolve 0.1433 gm. of KH_2PO_4 in distilled water, transfer quantitatively to a 1000-cc. volumetric flask, add 0.5 cc. of toluene and dilute to the mark. This solution contains 100 p.p.m. PO_4. Take 10 cc. of this solution and dilute to 1000 cc. in a volumetric flask to obtain a solution of 1.0 p.p.m. PO_4. All standards are prepared from this solution. To prepare a standard containing 0.10 p.p.m. PO_4 dilute 10 cc. of the second phosphate solution to 95 cc., add 2.0 cc. of reagent A and 6 drops of reagent B and dilute to 100 cc.

Procedure for the determination of inorganic phosphates

Place 50 cc. of the clear solution or extract in a 100-cc. Erlenmeyer flask. The solution should not contain more than 1.0 p.p.m. PO_4. If the concentration is greater than 1.0 p.p.m. use only enough solution so that when diluted to 50 cc. the concentration will be about 0.50 p.p.m. PO_4. Add from a burette 1.0 cc. of reagent A and while shaking the flask add 3 drops of reagent B to develop the blue color.

A series of standards should be prepared at the same time as the unknown. This is conveniently done by placing 5, 10, 25, and 50 cc. of the phosphate solution in 150-cc. Erlenmeyer flasks, add water to make about 97 cc., then add 2 cc. of reagent A and 6 drops of reagent B. Dilute to exactly 100 cc. in a graduated cylinder or volumetric flask.

After both the standard and unknown have stood 5 minutes for the color to develop, compare the unknown with the standard of approximately the same color intensity. When the color intensity is rather high, about that obtained at a concentration of 0.50 p.p.m. PO_4, the comparison is best made with a colorimeter. When only a slight color is developed the comparison is best made with Nessler tubes.

Procedure for the determination of total phosphorus

Place 50 cc. of the soil extract or solution add 1.0 cc. of normal $Mg(NO_3)_2$ in a 75-cc. porcelain casserole, and evaporate to dryness on a hot plate or steam bath. Ignite over a Bunsen burner to destroy all organic matter. After cooling add 5 cc. of dilute HCl, 1:20, and place on a hot plate to dissolve the residue. Permit the acid to evaporate to a volume of about 1.0 cc. If it should go to dryness add more acid to bring the residue into solution again. When solution is complete, except for silica in some instances, add water, transfer to a 50-cc. graduated cylinder and dilute to 49 cc. Pour the contents of the cylinder into a 100-cc. Erlenmeyer flask and add the reagents to develop the blue color. At the same time prepare standards as given under the procedure for inorganic phosphate. After five minutes, compare the unknown with the standard of approximately the same concentration.

To determine organic phosphorus make determinations of total and of inorganic phosphorus. Subtract the inorganic phosphorus from the total to obtain organic phosphorus.

Discussion of method

It is essential that the solution to be tested be colorless. If the solution or extract is slightly colored with organic matter the determination of inorganic phosphorus can be estimated with a fair degree of accuracy. Some soils, however, give solutions that are so colored that it is impossible to determine inorganic phosphorus. Since the residue is ignited for the determination of total phosphorus, the final solution is always colorless.

Development and permanency of the blue color. Experiments have shown that the maximum color will be developed in five minutes. If the acidity is increased to greater extent than recommended in the procedure for total phosphorus the rate of development of the color will be retarded. Too great an acidity, as will be shown later, will prevent the development of the maximum color.

After the color is developed it remains unchanged for a considerable period. Careful experiments have shown that under the usual laboratory conditions there was no color change within one hour. In these experiments the flasks were not placed in direct sunlight. It seems, therefore, that the readings may be made any time from five minutes to one hour after the reagents have been added.

TABLE 1

Influence of added acid on the colorimetric determination of phosphorus

AMOUNT OF 1:20 HCl ADDED TO 100 CC. SOLUTION	CONCENTRATION OF PO_4 IN SOLUTION		INFLUENCE OF ADDED ACID
	Theoretical	Determined	
cc.	*p.p.m.*	*p.p.m.*	*p.p.m.*
None	0.250	0.247	....
1	0.250	0.246	−0.004
2	0.250	0.259	+0.009
5	0.250	0.260	+0.010
10	0.250	0.257	+0.007
20	0.250	0.206	−0.044
25	0.250	0.160	−0.090

TABLE 2

Influence of different concentrations of silica on the colorimetric determination of phosphorus

	NO PHOSPHORUS IN SOLUTION			PHOSPHORUS IN SOLUTION		
CONCENTRATION OF SiO_2	Concentration of PO_4		Increase due to SiO_2	Concentration of PO_4		Increase due to SiO_2
	Theoretical	Determined		Theoretical	Determined	
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
25	None	0.00	0.00	0.10	0.10	0.00
50	None	0.00	0.00	0.10	0.10	0.00
100	None	0.01	0.01	0.10	0.10	0.00
200	None	0.03	0.03	0.10	0.12	0.02
500	None	0.06	0.06	0.10	0.15	0.05
1,000	None	0.09	0.09	0.10	0.22	0.12
2,500	None	0.18	0.18	0.10	0.32	0.22

Influence of Acidity. The determination must be made at a rather definite acidity. The proper acidity is produced by the sulfuric acid in reagent A. If the acidity is materially reduced a color will be produced even if phosphorus is not in solution (5). If the acidity is increased too much the maximum color will not be produced. Consequently, the amount of acid used to take up the residue after ignition must be carefully regulated. In order to determine the amount of acid that might be safely used the following experiment was made. Different amounts of 1:20 hydrochloric acid were added to a known phosphate solution and the color was developed. After the solution had stood fifteen minutes the color was compared with that of a standard prepared in the usual manner. The results are given in table 1 and show that the addition of as much as 10 cc. of 1:20 HCl had no significant influence on the determination. The addition of 20 cc. of the dilute acid produced a low result.

Five cubic centimeters of 1:20 acid is recommended since that amount does not influence the color development and is enough to bring the residue into solution. In taking up the residue, approximately one-half of the acid is expelled before water is added to the casserole.

Influence of various salts. A study of the influence of various salts shows that none of the salts commonly found in the soil solution interferes with the determination when present in concentrations comparable to those found in the soil solution. Arsenates give the same color reaction as phosphates but are not ordinarily found in the soil solution.

TABLE 3

Phosphate content of a displaced solution as determined after evaporation and ingnition with nitric acid and different amounts of magnesium nitrate

EVAPORATED WITH	2 cc. HNO_3	1.0 cc. N $Mg(NO_3)_2$	2.0 cc. N $Mg(NO_3)_2$	5.0 cc. N $Mg(NO_3)_2$
PO_4 determined........................p.p.m.	0.475	0.480	0.490	0.480

Silica will produce a blue color when present in sufficiently large quantities. Small amounts of silica such as are ordinarily found in soil solutions do not produce the color reaction. This is shown by the following experiment. Solutions were prepared containing from 0 to 2500 p.p.m. SiO_2. To one series of solutions 0.10 p.p.m. PO_4 was added. No phosphorus was added to the other series. The reagents were added to both series and the color developed was compared with that of a standard prepared in the usual manner. The results are given in table 2.

When there was no phosphorus in solution a slight color could be detected when 100 p.p.m. of SiO_2 was in solution. When a small amount of phosphorus was added the increase in color could not be detected until there was 200 p.p.m. of SiO_2 in solution. Florentin (7) obtained a slight color with 49 p.p.m. of SiO_2. These data show that there is little probability that the silica in the soil solution would influence the determination of phosphorus. This is one of the chief advantages of this method over the yellow colorimetric method.

Influence of ignition. It is well known that there may be a loss of phosphorus on strong ignition if there is a deficiency of base. In the procedure previously recommended for total phosphorus (8) the solution was evaporated with a small amount of nitric acid and the residue ignited over a Bunsen burner without the addition of a base. The bases normally present in the soil solution of even the most acid soils were assumed to be sufficient to prevent loss of phosphorus. In order to study this matter, phosphorus was determined in several soil solutions and extracts by the old procedure modified by the addition of magnesium nitrate. Table 3 gives the results of a typical series of determinations on a displaced solution. These results together with a large number of a similar nature indicate that there is little or no loss of phosphorus on evaporation with nitric acid and subsequent ignition. In a few instances the results with soil extracts indicated a slight loss of phosphorus when magnesium nitrate was not added. In no case was the loss more than about ten per cent of the total. In order to avoid possible loss and to facilitate the ignition the use of 1.0 cc. of N $Mg(NO_3)_2$ is recommended.

Comparison with volumetric method. The method has been compared with the regular volumetric method for the determination of phosphorus. The comparison was made on soil extracts, prepared from a series of plots which had received different phosphate fertilization. The data given in table 4 show that the agreement between the results of the two methods is satisfactory.

TABLE 4

Phosphate concentration of 1:5 soil extracts as determined volumetrically and colorimetrically

FERTILIZATION PER ACRE WITH ACID PHOSPHATE	PO_4 IN SOIL EXTRACT	
	Volumetric method	Colorimetric method
pounds	*p.p.m.*	*p.p.m.*
None	0.059	0.056
300	0.102	0.084
600	0.188	0.190
900	0.442	0.449
1,200	0.643	0.636

Organic and inorganic phosphorus in the displaced solution and soil extracts. The method has been used rather extensively by Pierre and Parker (9) in a study of the content of organic and inorganic phosphorus in the displaced solution and in soil extracts. The data in their paper serve to illustrate the nature of the results obtained with the method.

THE COLORIMETRIC METHOD OF FISKE AND SUBBAROW

The colorimetric method of Fiske and Subbarow or very similar methods have been used by biological chemists for several years. Bell and Doisy (3), Briggs (4), and Fiske and Subbarow (6) have studied the method and offered various modifications. The procedure of Fiske and Subbarow seems to offer several advantages over the older procedures. The writers have used their procedure, and find it very satisfactory for the determination of both organic and inorganic phosphorus at concentrations above 1.00 p.p.m. PO_4. The method may be used in plant analysis or in the determination of phosphorus in acid extracts of soils. When used in such work, if the color comparison is made by means of a colorimeter, the method gives very satisfactory results. Table 6 gives the results of the analysis of some plant tissue using the volumetric and colorimetric methods. In every instance the agreement between the results obtained by the two methods is good. In a further study the two colorimetric methods were compared, soils extracts that were unusually high in phosphorus being used. The data obtained by the two methods agreed satisfactorily. These results lead to the conclusion that the procedure recommended by Fiske and Subbarow is well adapted for use in several different phases of soil investigations. The following procedure is that recommended by Fiske and Subbarow.

TABLE 5

The phosphorus content of plant tissue as determined volumetrically and colorimetrically by the Fiske and Subbarrow method

SAMPLE NUMBER	PO_4 CONTENT OF SAMPLE	
	Volumetric method	Colorimetric method
	mgm.	*mgm.*
1	25.85	25.35
2	28.47	27.95
3	25.25	24.70
4	19.40	19.78
5	23.75	24.60
Average of 12 samples.......	25.01	24.93

Solutions needed

10 normal sulfuric acid. 450 cc. of concentrated arsenic-free sulfuric acid added to 1300 cc. of water.

Molybdate. 2.5 per cent ammonium molybdate in 5 *N* sulfuric acid. Dissolve 25 gm. of the salt in 200 cc. of water. Rinse into a liter volumetric flask containing 500 cc. of 10 *N* sulfuric acid. Dilute to mark with water and mix.

Standard phosphate solution. Dissolve 0.1433 gm. of KH_2PO_4 in distilled water, transfer quantitatively to a 1000 cc. volumetric flask, add 0.5 cc. of toluene and dilute to the mark. One cubic centimeter of this solution diluted to 100 cc. gives a solution containing 1.0 p.p.m. PO_4. A standard containing 5 p.p.m. PO_4 is satisfactory for most work.

15 per cent sodium bisulfite. The solution must be free from turbidity before it can be used. Freshly prepared sodium bisulfite solutions may not filter clear, in which case 2 or 3 days standing (before filtering) will be necessary. Keep well stoppered.

20 per cent sodium sulfite. Because it is likely to be of better quality, the authors prefer to use the crystalline sulfite ($Na_2SO_3 \cdot 7H_2O$). Dissolve 200 gm. of this in 380 cc. of water. Remove any suspended matter by filtration, and keep stoppered.

0.25 per cent aminonaphtholsulfonic acid. Dissolve 0.5 gm. of the dry powder [see (6, page 388) for method of preparation] in 195 cc. of 15 per cent sodium bisulfite, add 5 cc. of 20 per cent sodium sulfite, stopper, and shake until dissolved. If the bisulfite solution is old, more than 5 cc. of sulfite will be needed —in that event add more sulfite, 1 cc. at a time, shaking after each addition, until solution is complete. This reagent can be prepared in a few minutes (the powder need not be very accurately weighed), and if not left exposed to the air it should keep about 2 weeks. The solution is more stable the higher its acidity, hence no more sulfite should be added than is needed to dissolve the reducing agent.

Procedure for inorganic phosphate

Measure into a 100 cc. volumetric flask enough solution to contain between 0.2 and 1.5 mgm. inorganic PO_4. Add water to bring the volume to about 70 cc., followed by 10 cc. of 2.5 per cent ammonium molybdate made up in 5 *N* sulfuric acid, and add 4 cc. of 0.25 per cent aminonaphtholsulfonic acid. After the addition of each reagent, the solution should be mixed by gentle shaking.

At the same time transfer to a similar flask 5 cc. of the standard phosphate solution (containing 0.5 mgm. PO_4), 65 cc. of water, and the same reagents that were added to the unknown. Dilute the contents of each flask to the mark, mix, and compare in the colorimeter after 5 minutes. Calculate the concentration of PO_4 in the unknown.

SUMMARY

A study was made of the Deniges and Fiske and Subbarow methods for the colorimetric determination of phosphorus in the soil solution and soil extracts. The procedure is given for the determination of total and inorganic phosphorus.

The method of Deniges is more sensitive than that of Fiske and Subbarow, and is, therefore, recommended for most work with soil extracts and the soil solution. Data are given showing that moderate amounts of silica do not influence the determination. The Fiske and Subbarow method may be useful in plant analysis or for the determination of phosphorus soluble in dilute acids.

REFERENCES

(1) Atkins, W. R. G. 1923 The phosphate content of fresh and salt waters in its relationship to the growth of the algal plankton. *Jour. Marine Biol. Assoc.* 13: 119–150.

(2) Atkins, W. R. G. 1924 The rapid determination of available phosphate in soil by the coeruleo—molybdate method of Deniges. *Jour. Agr. Sci.* 14: 192–197.

(3) Bell, R. D., and Doisy, E. A. 1920 Rapid colorimetric methods for the determination of phosphorus in urine and blood. *Jour. Biol. Chem.* 44: 55–67.

(4) Briggs, A. P. 1922 A modification of the Bell-Doisy phosphate method. *Jour. Biol. Chem.* 53: 13–16.

(5) Denigès, G. 1920 Reaction de coloration extremement sensible des phosphates et des arseniates. Ses applications. *Compt. Rend. Acad. Sci.* (Paris). 171: 802–804.

(6) Fiske, C. H., and Subbarow, Y. 1925 The colorimetric determination of phosphorus. *Jour. Biol. Chem.* 66: 375–400.

(7) Florentin, D. 1921 Determination de phosphate dans l'eau. *Ann. Chim. Analyt.* 3: 295–296.

(8) Parker, F. W. 1925 The absorption of phosphate by Pasteur-Chamberland filters. Soil Sci. 20: 149–158.

(9) Pierre, W. H., and Parker, F. W. 1927 Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and the availability of the organic phosphorus to plants. Soil Sci. 24: 119–128.

(10) Taylor, A. E., and Miller, C. W. 1914 On the estimation of phosphorus in biological material. *Jour. Biol. Chem.*, 18: 215–224.

(11) Wu, H. 1920 Contribution to the chemistry of phosphomolybdic acids, phosphotungstic acids, and allied substances. *Jour. Biol. Chem.* 43: 189–220.

SOIL PHOSPHORUS STUDIES: II. THE CONCENTRATION OF ORGANIC AND INORGANIC PHOSPHORUS IN THE SOIL SOLUTION AND SOIL EXTRACTS AND THE AVAILIBILITY OF THE ORGANIC PHOSPHORUS TO PLANTS[1]

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Since the development of the displacement method of obtaining the soil solution, several investigators (1, 2, 6, 8) have made studies on the phosphorus content of the displaced solution. In none of these studies, however, has the distinction been made between the organic and the inorganic phosphorus in the solution, nor has this differentiation been made in studies of the phosphorus content of water extracts of soils. The development of the blue colorimetric method (5) for the determination of small quantities of phosphate makes possible the quantitative determination of organic and inorganic phosphorus in soil solutions and soil extracts.

Since preliminary studies indicated that a considerable portion of the phosphorus in soil solutions is organic, it seemed very desirable to study the relative availability of organic and inorganic phosphorus to plants. Consequently, the present investigation was made for a twofold purpose: first, to study the concentration of organic and inorganic phosphate in the displaced solution and in the water extract of a number of representative soils; second, to study the relative availability to plants of the two forms of phosphorus, as indicated by studies on absorption and plant growth.

EXPERIMENTAL

Organic and inorganic phosphorus in displaced soil solutions and in soil extracts

Twenty-one representative soils were secured from nine states in the South and Middle West.[2] These soils were chosen to represent a wide range in texture, reaction, and content of organic matter. Table 1 gives the soil type, the content of total phosphorus, and the phosphorus soluble in 0.2 N nitric acid.

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[1] Published with the permission of the Director of the Alabama Agricultural Experiment Station.

[2] The authors wish to express their appreciation to the following men for furnishing soil samples from their respective states; Dr. F. J. Alway and Dr. R. M. Pinckney, Minnesota; Prof. E. Truog, Wisconsin; Dr. F. C. Bauer, Illinois; Prof. E. J. Kinney, Kentucky; Prof. Martin Nelson, Arkansas; Prof. W. E. Ayres, Mississippi; Prof. W. J. Davis, Georgia; and Prof. T. S. Buie, South Carolina.

The fourth column of the table gives the crop response to phosphate fertilization. In many instances the crop response of the soil had been determined by field experiments; in other instances, by greenhouse experiments. It should be understood that the relative crop response for the different soils indicated in the table is only an approximation. The response would of course vary with the crop indicator and numerous other factors. There can be no doubt, however, that the study includes a number of soils that do not respond to phosphate fertilization and a number that give a large response. Where there was considerable doubt as to the phosphate response it was recorded as "unknown."

TABLE 1

Description and phosphorus content of soils used in the investigation

SOIL NUMBER	SOIL TYPE	LOCATION	CROP RESPONSE TO PHOSPHATE FERTILIZATION	TOTAL PHOSPHORUS PER ACRE	PHOSPHORUS PER ACRE SOLUBLE IN 0.2 *N* HNO_3	TOTAL PHOSPHORUS SOLUBLE IN 0.2 *N* HNO_3
				pounds	*pounds*	*per cent*
1	Greenville sandy loam	Alabama	Slight	490	19	3.9
2	Norfolk sandy loam	Alabama	None	460	54	11.7
3	Orangeburg sand	Alabama	Good	274	8	2.9
4	Ruston sand	Alabama	Good	240	9	3.7
5	Cecil clay loam	Alabama	Unknown	914	24	2.6
6	Portsmouth sandy loam	Alabama	Unknown	500	37	7.4
7	Norfolk sandy loam	Alabama	Unknown	420	28	6.7
8	Decatur silt loam	Alabama	Good	674	7	1.0
9	Silt loam	Mississippi	None	1,240	459	37.0
10	Tifton sandy loam	Georgia	Medium	554	92	16.6
11	Cecil sandy loam	South Carolina	Unknown	654	90	13.7
12	Lintonia fine sandy loam	Arkansas	Unknown	1,110	46	4.1
13	Hampstead silt loam	Minnesota	None	1,564	63	4.0
14	Webster silty clay	Minnesota	Medium	1,340	147	11.0
15	Maury silt loam	Kentucky	None	5,644	697	12.3
16	Miami silt loam	Wisconsin	None	1,334	226	16.9
17	Colby silt loam	Wisconsin	None	1,434	163	11.3
18	Colby silt loam	Wisconsin	None	1,074	26	2.4
19	Grundy silt loam	Illinois	None	1,100	24	2.2
20	Gray silt loam	Illinois	None	1,024	137	13.4
21	Gray silt loam	Illinois	Slight	694	36	5.2

As the table shows, the total phosphorus content of the soils varied from 240 pounds per acre in a Ruston sand from Alabama to 5644 pounds per acre in the Maury silt loam from Lexington, Kentucky. The variation in phosphorus soluble in 0.2 *N* nitric acid is very striking, ranging from 7 to 697 pounds per acre. Only 1 per cent of the total phosphorus in soil 8, Decatur silt loam, was soluble in dilute acid. On the other hand, 37 per cent of the total phosphorus in soil 9, a silt loam from the Delta substation of the Mississippi Agricultural Experiment Station, was soluble in dilute acid. These data show that the twenty-one soils represent a very wide range of conditions with respect to total phosphorus, phosphorus soluble in dilute acids, and the crop response to phosphate fertilization. It would be expected, therefore, that the soils would show a wide variation in the organic and inorganic phosphate content of the displaced solution and 1:5 soil extract.

TABLE 2

Data on the displaced solution and 1:5 soil extract of the soils indicated

SOIL NUMBER	SOIL TYPE	MOISTURE CONTENT	DISPLACED SOLUTION			1:5 EXTRACT		
			Specific resistance	Inorganic PO_4	Organic PO_4	Inorganic PO_4	Organic PO_4	H-ion concentration
		per cent	*ohms*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*pH*
1	Greenville sandy loam	11.2	420	0.03	0.23	0.06	0.10	5.65
2	Norfolk sandy loam	9.4	1,212	0.14	0.54	0.38	0.26	5.67
3	Orangeburg sand	9.2	1,580	0.02	0.28	Trace	0.13	5.57
4	Ruston sand	7.0	2,020	Trace*	0.38	Trace	0.09	5.40
5	Cecil clay loam	10.0	483	Trace	0.42	0.05	0.05	5.80
6	Portsmouth sandy loam	8.1	729	Trace	0.24	Trace	0.12	4.40
7	Norfolk sandy loam	12.9	126	0.04	0.60	0.03	0.17	5.20
8	Decatur silt loam	13.1	1,175	0.05	0.15	Trace	0.06	5.55
9	Silt loam	14.6	572	0.30	0.43	0.85	0.40	6.00
10	Tifton sandy loam	8.4	152	0.32	0.60	1.72	0.57	5.65
11	Cecil sandy loam	14.6	93	0.06	0.90	0.11	0.14	5.60
12	Lintonia fine sandy loam	19.8	668	Trace	0.30	0.17	0.07	6.00
13	Hempstead silt loam	20.6	653	Trace	0.51	0.35	0.45	5.40
14	Webster silty clay	14.5	256	0.04	0.88	0.17	0.23	7.70
15	Maury silt loam	18.5	680	0.08	0.56	1.58	0.26	5.85
16	Miami silt loam	20.2	274	7.26†	....	4.80	0.64	6.25
17	Colby silt loam	21.4	548	0.03	0.26	Trace	0.25	5.50
18	Colby silt loam	19.6	1,083	0.03	0.37	Trace	0.22	5.95
19	Grundy silt loam	21.2	585	0.04	0.68	0.03	0.15	5.20
20	Gray silt loam	18.8	243	0.35	0.89	0.98	0.62	6.15
21	Gray silt loam	17.4	216	0.20	0.18	0.19	0.11	6.30
Average		....	.....	0.09	0.47	0.35	0.22	....

* Results are recorded as trace when the concentration is less than 0.02 p.p.m. In some instances the solution was apparently phosphorus free.

† Figure is for total phosphorus. Results for this soil are not included in the average.

After the soils were brought to the laboratory, water was added to bring them to a moisture content satisfactory for displacement. After standing 24 hours, each was thoroughly mixed and allowed to stand in a covered jar for at least 48 hours before the soil solution was displaced. The displacement method as modified by Burd and Martin (1) was used to obtain the soil solution. The specific resistance of the solution was determined at the temperature of 25°C.

The soil extracts were prepared from air-dry soil by the collodion sack method (7). The H-ion concentration of the extract was determined colorimetrically by the procedure described in a previous paper (7).

The content of organic and inorganic phosphorus was determined by the procedure recommended by Parker and Fudge (5).

Table 2 gives the data obtained from a study of the displaced solutions and the 1:5 soil extracts. As indicated by the specific resistance, the salt content of the soil solutions varied widely, from a resistance of 93 ohms to 2022 ohms. The corresponding figures for total solids before ignition are 9,430 and 540 p.p.m. The reaction of the extracts varied from pH 4.40 to 7.70, but the reaction of most of the soils was between pH 5.0 and 6.0.

The data for phosphorus in the displaced solution show that nearly all soils give solutions having a very low concentration of inorganic phosphate. The maximum concentration, with one exception, was 0.35 p.p.m. The displaced solution from soil 16 contained 7.26 p.p.m. total phosphate. Although inorganic phosphate was not determined, the data on the water extract make it seem probable that the inorganic phosphate content was approximately 6.0 p.p.m. This soil had been heavily fertilized and would probably be considered a "garden soil." Several of the very productive soils give solutions with a very low concentration of inorganic phosphate. For instance, soil 15 from Lexington, Kentucky, and soil 13 from Aledo, Illinois, are both very productive, having an average corn yield of more than fifty bushels an acre, yet the displaced solution from these soils contained only 0.08 and 0.04 p.p.m. inorganic PO_4. Soils 17 and 18 are considered very fertile and do not respond to phosphate fertilization; yet their displaced solution contained only 0.03 p.p.m. inorganic phosphate. A careful study of all the data indicates that there is only a slight correlation between the concentration of inorganic phosphate in the displaced solution and the crop response to phosphate fertilization when all soils are considered. These data and some unpublished results show that a better correlation is secured when the comparison is limited to soils of the same or similar types.

The concentration of organic phosphate in the displaced solution varied from 0.15 p.p.m. to 0.90 p.p.m. PO_4 with an average for the twenty soils of 0.47 p.p.m. This represents 84 per cent of the phosphorus in the displaced solution and shows that in most soils a large part of the phosphate in solution is in the organic form. In only one soil, number 16 excepted, was there as much inorganic as organic phosphorus.

The results for phosphorus in the displaced solution show that the solutions from nearly all soils contain at least 0.20 p.p.m. total PO_4. This result differs from that of Parker and Tidmore (6) in that their data indicate that some soils give solutions much lower in total phosphate. This difference is probably due to the fact that they dialyzed all of their solutions and, as shown by Pierre and Parker (7), the collodion sack may absorb a portion of the phosphate. The resulting error is probably rather large when the solutions contain only small amounts of phosphate, most of it in the organic form. The error, however, was probably not large enough to influence the conclusions drawn from the work. There was no such error in their determination of phosphorus in the soil extract. It should be noted, however, that in their work on soil extracts only inorganic phosphate was determined.

Burd and Martin (1) determined the phosphorus content of the displaced solution of several California soils. The content of total PO_4 varied from 1.1 to 12.5 p.p.m. All of the soils studied were slightly alkaline and apparently relatively high in water-soluble phosphorus. Most of the phosphate in the displaced solution from such soils is probably in the inorganic form.

The inorganic phosphate content of the soil extracts varied from a trace in several soils to 4.80 p.p.m. in the extract from soil 16. Three soils gave extracts containing more than 1.0 p.p.m. inorganic phosphate. Here, as in the displaced solution, several productive soils that do not respond to phosphate fertilization have only a trace of inorganic phosphate in their extracts. Apparently, there is little correlation between water-soluble phosphate and the crop response to phosphate on different soils. Such a correlation would probably be found if the study were limited to soils of the same or similar types.

Probably one of the most interesting studies that can be made with the data of table 2 is to compare the results obtained with the displaced solutions and the soil extracts. Such a comparison shows that in many instances the soil extract contains considerably more inorganic phosphate than the displaced solution. The average for the twenty soils is 0.09 p.p.m. in the displaced solution and 0.35 p.p.m. in the water extract. The organic phosphate gives just the opposite result, the average concentration in the displaced solution being more than twice the concentration in the extract. Apparently, the solubility of the inorganic phosphate is much greater than that of the organic. Possibly a better explanation of the results would be to assume that the salts of the soil solution depress the solubility of the inorganic phosphate more than that of the organic phosphate. Then as a result of dilution with water the high solubility of the inorganic phosphate is made more apparent. The data indicate that although the concentration of inorganic phosphate in the soil solution is very low, the solubility of the inorganic soil phosphate is such that the concentration can probably be maintained nearly constant. The organic phosphate, on the other hand, is relatively much higher in the displaced solution but has a low solubility in many soils.

Availibility of the organic phosphorus

Since the data in table 2 show that most of the phosphorus in the soil solution is organic it is important to determine whether the organic phosphorus can be absorbed by plants. In the past apparently it has been assumed that all of the water-soluble phosphate is inorganic. At least the assumption has been made that all water-soluble phosphorus is available to plants (3, 6). It is essential that the validity of this assumption be determined since considerable attention has been given to the relation of the composition of the displaced solution to the mineral nutrition of the plant.

In order to study the relative availability of the two forms of phosphorus several absorption experiments were carried out as follows: Corn, soybean, and buckwheat plants were grown in culture solutions containing only enough phosphate to support a good vigorous growth. After the plants had attained some size and were making rapid growth, they were placed in culture vessels containing either a soil extract or a quantity of displaced solution. Organic and inorganic phosphates were determined in the soil extract or solution before the plants were placed in it. After the plants had been in the solution 24 hours it was again analyzed for organic and inorganic phosphorus. At the same time determinations were made on blanks—solution in which no plants were growing. The results of the experiments are given in table 3. Since the concentration of organic and inorganic phosphate in the blanks did not change during 24 hours the final results are not given in the table.

TABLE 3

Data on the absorption of the inorganic and organic phosphorus of soil extracts and a soil solution by different plants*

NUMBER	PLANT		PO_4 IN CULTURES AT BEGINNING		PO_4 IN CULTURES AFTER 24 HOURS		ABSORPTION OF PO_4 IN 24 HOURS	
	Kind	Age	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic
		weeks	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	Buckwheat	5	0.52	0.40	0.23	0.43	0.29	0.00
2	Soybeans	2	0.52	0.40	0.04	0.38	0.48	0.02
3	Soybeans	6	0.68	0.17	Trace	0.16	0.68	0.01
4	Corn	4	0.68	0.17	Trace	0.18	0.68	0.00
5	Corn†	8	0.18	0.26	Trace	0.30	0.18	0.00
6	Corn	4	0.35	0.18	0.02	0.20	0.33	0.00
7	Corn	4	0.12	0.28	Trace	0.32	0.12	0.00

* Culture 7 was made up of a composite of several displaced soil solutions.

In cultures 1, 2, and 5 there was one plant per 500 cc. of soil extract, in cultures 3 and 4 there was one plant in 2000 cc., whereas in cultures 6 and 7 there was one plant per 1000 cc. Some, but not all, of the above cultures were in duplicate.

† There was only a trace of inorganic PO_4 in the cultures after the plants had remained in them for five hours.

The data in table 3 show that corn, soybeans, and buckwheat absorb inorganic phosphate very readily. Practically all of the inorganic phosphorus was absorbed in 24 hours except in the case of buckwheat. In several of the cultures determinations made after 6 hours showed that the inorganic phosphorus was almost completely absorbed in that period of time. On the other hand, the data show that none of the organic phosphate was absorbed by any plant in the 24-hour period. In cultures 3, 4, 6, and 7, the concentration of organic phosphorus was the same after 48 hours as at the beginning of the experiment.

The data seem to show rather conclusively that the organic phosphorus, as such, in the displaced solution or in the soil extract is not available to plants. Apparently, the plant absorbs only inorganic phosphate. The significance of this conclusion will be discussed later in the paper.

In addition to the absorption experiments, another experiment was made in order to study further the availability of water-soluble organic phosphorus to the corn plant. In this experiment corn was grown for a period of 41 days in soil extracts some of which contained only organic phosphorus whereas others contained both organic and inorganic phosphorus. Seven soils, from field plots of known phosphate fertilization, were used in the experiment. The cultures were so arranged that the phosphate content of the extract was rather effectively renewed by more phosphate coming into solution from the soil. The detailed procedure follows:

One hundred grams of dry soil were placed in each of twenty 500-cc. collodion sacks. Water was added to make a thin mud and the sacks were closed by tying with a soft cotton string. The 20-sacks were then placed in a 1-gallon earthenware jar and 3000 cc. of a phosphorus-free nutrient solution was added to fill the jar. A glass tube entered the jar through a rubber stopper near the bottom. This provided a means of aeration and of stirring the extract, thereby hastening diffusion from the soil. Aeration was obtained by connecting all jars with an air pump, which was operated by an automatic time switch. The switch was adjusted to operate the pump for a period of 3 minutes at intervals of 1 hour. The bubbling in the jars was very rapid during the 3 minutes and seemed to afford very good aeration and stirring.

The organic and inorganic phosphate content of the extract or culture solution was determined twice a week during the growing period. As the plants grew and absorbed phosphorus the concentration of inorganic phosphate decreased. The concentration of organic phosphorus remained nearly constant. The phosphate content indicated in the accompanying table is the average of sixteen determinations made at regular intervals during the growing period.

All cultures were in duplicate with two plants to each culture. The corn was harvested 41 days after planting and the dry weight and phosphorus content of the plants were determined (table 4).

The first three soils gave extracts that contained considerable organic phosphorus but not a determinable amount of inorganic phosphate. The corn made practically no growth in these extracts. Although a phosphorus-free culture solution was not included in this series, other work (table 5) shows that such a culture produces two plants weighing about 2.0 gm. and containing approximately 5.0 mgm. of phosphate. Apparently, therefore, the plants in the first three cultures made no more growth than they would have made in a phosphorus-free solution. The growth in the last four cultures, which all contained some inorganic phosphate, was closely correlated with the average inorganic phosphate content of the extract, and was even more closely correlated with the amount of phosphate absorbed. The organic phosphate content of the different extracts was nearly the same, the average for the first three extracts being 0.15 p.p.m. and the average for the last four, 0.18 p.p.m. PO_4. It is evident from this experiment that corn will not grow on organic phosphate at the concentration found in these soil extracts. The same concentration of inorganic phosphate produced good growth. This result confirms the conclusion drawn from the absorption studies; namely, the organic phosphate in the soil solution is not absorbed by the plants used in these experiments.

DISCUSSION

The experimental results bring out two important facts: First, most of the phosphorus in the displaced solutions is in the organic form, the concentration of inorganic phosphate in the displaced solution being very low; second, the water-soluble organic phosphate is not absorbed by plants and is consequently unavailable to the plant as long as it remains in the organic form.

TABLE 4

Dry weight and phosphorus content of corn grown in soil extracts of the phosphorus content indicated

SOIL	PHOSPHATE TREATMENT	PO_4 IN SOIL EXTRACT*		DRY WEIGHT OF TWO PLANTS	PO_4 CONTENT OF PLANTS	
		Inorganic	Organic			
		p.p.m.	*p.p.m.*	*gm.*	*per cent*	*mgm.*
Silt loam	None	Trace	0.14	1.70	0.29	5.0
Sandy loam	None	Trace	0.16	2.22	0.31	6.9
Black clay loam	None	Trace	0.15	2.35	0.28	6.5
Silt loam	None	0.02	0.14	3.97	0.29	11.3
Sandy loam	Acid phosphate	0.04	0.18	7.22	0.32	23.0
Silt loam	Bone meal	0.08	0.18	6.45	0.35	22.5
Silt loam	Acid phosphate	0.19	0.21	14.50	0.34	48.8

* Each figure is the average of 16 determinations made at regular intervals during the growing period.

The unavailability of the organic phosphate does not mean that such phosphates may not be made available to the plant by biological agencies within the soil. It is probable that in all soils, organic phosphates are being decomposed and the phosphorus is being made available to the plants as a result of bacterial action. The rapidity with which the organic phosphate is made available depends on many factors, one of the most important being the nature of the organic matter. The data of table 4 indicate that the rate of conversion is very slow in those soils, for during the growing period of 41 days very little, if any, of the organic phosphate became available to the corn. The slow rate of conversion is further indicated by the fact that in the displaced solution of most of the twenty-one soils studied more than seventy-five per cent of the phosphorus was organic. If the organic phosphate was being rapidly changed into the inorganic form this would probably not be the case. Apparently, therefore, the concentration of organic phosphate in the soil solution and soil extract represents the solubility of relatively insoluble and stable organic matter that contains a small percentage of phosphorus.

In studies on the relation between the composition of the soil solution and the mineral nutrition of the plant apparently the assumption has been made that all water-soluble phosphate is available to the plant (3, 6). That such an assumption is erroneous is shown by the experimental data in tables 3 and 4. Consequently, when such studies are being made only the concentration of inorganic phosphates should be considered. Since the concentration of inorganic phosphate in many soils is so very low as compared to the content of total phosphorus, this distinction may make a very decided difference in the conclusions arrived at from plant nutrition studies.

SUMMARY

The content of organic and inorganic phosphate was determined in the displaced solution and 1:5 water extracts of 21 soils from 9 states. Absorption and growth studies were then made to determine the availability to plants of the water-soluble organic phosphate. The results of the investigation may be summarized as follows:

1. In the displaced solution from 20 soils, the average content of inorganic phosphate was 0.09 p.p.m., and the average content of organic phosphate was 0.47 p.p.m.
2. The 1:5 soil extracts of the same soils gave an average concentration of 0.35 p.p.m. inorganic phosphate and 0.22 p.p.m. organic phosphate.
3. It was shown by absorption experiments that plants would not absorb organic phosphate from soil extracts or the displaced solution. In the same experiment the plants absorbed all of the inorganic phosphate present.
4. In another experiment corn made no growth when organic phosphate in soil extracts was the only source of phosphorus, but made good growth in other soil extracts containing inorganic phosphate. The growth in the various extracts was almost proportional to the inorganic phosphate present.
5. The bearing of these results on problems in plant nitrition is briefly discussed.

REFERENCES

(1) Burd, J. S., and Martin, J. C. 1923 Water displacement of soils and the soil solution· *Jour. Agr. Sci.* 13: 265–295.

(2) Fudge, J. F. 1926 Private communication regarding work done at the Wis. Agr. Exp. Sta.

(3) Hoagland, D. R. 1925 Physiological aspects of soil solution investigations. *Hilgardia* 1: 227–257.

(4) Parker, F. W. 1927 Soil Phosphorus Studies: III. Plant growth and the absorption of phosphate from culture solutions of different phosphate concentrations. *Soil Sci.* 24: 129–146.

(5) Parker, F. W., and Fudge, J. F. 1927 Soil phosphorus studies: I. The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil Sci.* 24: 109–117.

(6) Parker, F. W., and Tidmore, J. W. 1926 The influence of lime and phosphatic fertilizers on the phosphorus content of the soil solution and of soil extracts. *Soil Sci.* 21: 425–442.

(7) Pierre, W. H., and Parker, F. W. 1926 The use of collodion sacks in obtaining clear soil extracts for the determination of the water-soluble constituents of soils. *Soil Sci.* 23: 13–33.

(8) Smith, A. M. 1926 Private communication regarding work done at the Wis. Agr. Exp. Sta.

SOIL PHOSPHORUS STUDIES: III. PLANT GROWTH AND THE ABSORPTION OF PHOSPHORUS FROM CULTURE SOLUTIONS OF DIFFERENT PHOSPHATE CONCENTRATIONS[1]

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Recent investigations in this laboratory (12, 14) have shown that the phosphate concentration of the displaced soil solution is usually very low. In the accompanying paper Pierre and Parker (14) show that the displaced solution from most of the soils studied contains less than 0.50 p.p.m. PO_4. A considerable proportion of the phosphorus in the solution was found to be organic and relatively unavailable to plants. In view of these results the question arises as to whether plants absorb all of their phosphorus from the solution and, if so, what concentration of phosphorus is necessary for satisfactory plant growth. If plants make satisfactory growth in culture solutions of approximately the same concentration as found in the displaced solution we can assume that the solid phase is relatively unimportant in plant nutrition, its only function being that of supplying various elements to the soil solution. If, however, plants will not grow in such dilute solutions it would be reasonable to explain plant growth in such soils by one or more of the following assumptions:

1. The displaced solution is not the true soil solution.
2. The plant roots exert a solvent action on the soil particles and thus bring more phosphorus into solution, producing a higher phosphate concentration at the absorbing surfaces.
3. As a result of a Donnan equilibrium, there is a higher concentration of phosphorus in the solution at the surface of the soil particles than in the displaced soil solution. The plant roots, being in very intimate contact with the soil particles, absorb most of their phosphorus from this more concentrated solution.

These points will be discussed in some detail after the presentation of experimental data on the growth of plants in solutions of different phosphate concentrations.

Hoagland and his associates have made a study of the phosphorus content of the soil solution and its relation to the phosphorus nutrition of plants. They first studied the composition of the displaced solution of several soils (1) and found that the phosphate content varied from 1.1 p.p.m. to 12.5 p.p.m. Hoagland and Martin (5) then grew plants in culture solutions of different phosphate concentrations, the lowest being 0.7 p.p.m. Very satisfactory growth of barley was secured at that concentration and in a second experiment, maximum growth was secured at a phosphate concentration of 1.1 p.p.m. As a result of these studies Hoagland (4) concludes that, "the concentration and composition of the soil solution from productive soils are of such a nature as to be adequate (for plant growth) apart from the solid phase of the soil." Unfortunately they did not study soils whose solution had lower concentrations of phosphate, nor did they study plant growth in more dilute solutions of phosphate.

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As a result of the recent studies in this laboratory it seemed that either Hoagland's conclusion was incorrect or that plants would grow in considerably more dilute phosphate solutions than were used by Hoagland and Martin. In an earlier paper (12) the writer and Tidmore expressed the opinion that plants would not make good growth in phosphate solutions of the concentration found in the displaced solution from many productive soils. In order to study these points in more detail, experiments were started to determine:

a. The concentration of inorganic phosphate essential for maximum plant growth.

b. The rate of absorption of phosphate from culture solutions of different phosphate concentrations.

EXPERIMENTAL PROCEDURE

The general plan of the various experiments was to grow plants in solution cultures at phosphate concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, 2.0, 5.0 and 10.0 p.p.m. A culture solution similar to one of Hoagland's (5, p. 372), was used in all the experiments. Table 1 gives the composition of the solution as used in experiments 1 and 4. The solution used in experiment 2 was one-half the concentration indicated in the table. Iron was added as ferric tartrate, but special consideration will be given to the method of supplying iron. Distilled water was used in the first and fourth experiments. In the second experiment rain water containing a slight trace of phosphorus was used in all cultures.

In all the solution culture experiments, an effort was made to maintain the phosphate concentration as nearly constant as possible. In an attempt to accomplish this, the plants were grown in rather large volumes of culture solution and the phosphate concentration was renewed once or twice a day. All cultures were in 7500-cc. glazed earthenware jars. In experiments 1, 3, and 4 there were two plants per culture but in experiment 2 only one plant was grown in a culture vessel.

The concentration of phosphate in the culture solution was determined every morning by the blue colorimetric method of Denigès (11). After the determinations were made, sufficient potassium acid phosphate was added to renew the original concentration of phosphorus. After the plants had attained some size, the concentration of phosphate was determined and renewed twice a day.

This procedure, together with the use of large volumes of solution, seemed to afford a good means of maintaining the phosphate concentration fairly constant. Subsequent data, however, will show that in some cultures of rather low phosphate concentration the method adopted did not satisfactorily maintain the concentration. The method has the advantage over some other methods that might be used, in that it gives some very significant data on the rate of absorption of phosphate.

After the plants were harvested, they were analyzed for phosphorus by the regular volumetric method.

Experiment 1—corn grown in culture solutions

Corn was germinated in quartz sand and transferred to culture solutions of different phosphate concentrations. Two vigorous seedlings were placed in each culture of 7500 cc. The solution had the composition indicated in table 1 and the phosphate concentration indicated in table 5. Ferric tartrate was added to give a concentration of 3.30 p.p.m. Fe. During the first two weeks the plants made satisfactory growth at phosphate concentrations of 1.0 p.p.m. or greater. At concentrations of less than 1.0 p.p.m. growth was poor and absorption of phosphate was rather slow. Once a week the entire solution was changed. It was noticed that with low concentrations of phosphate absorption was very rapid immediately following the change of solutions. Within twenty-four hours, however, the rate of absorption materially decreased although the concentration of phosphate was renewed. Accompanying this change in the rate of absorption there was a change in reaction from pH 5.0 to pH 6.2. It was also noticed that there was a considerable precipitate of iron in most of the cultures. These observations indicated that possibly the presence of iron was preventing the absorption of phosphate from solutions of low concentration by precipitating most of the phosphorus as colloidal iron phosphate. The colloidal phosphate remained in suspension and because of the acid reagent used in the phosphorus determination it produced the same analytical result that would have been secured if the phosphorus were in true solution.

TABLE 1

Composition of the culture solution

	K	Ca	Mg	NO_3	SO_4	NaCl	$MnSO_4$
Mgm. equivalent	2.98	7.60	3.25	10.70	3.25	..	...
P.P.M.	116	152	39	665	156	25	1.5

In order to determine whether part of the phosphate was precipitated as colloidal iron phosphate its diffusibility was determined. Small collodion sacks containing 100 cc. of water were placed in the culture solutions containing iron. At the same time sacks were placed in an iron-free solution. The reaction of both solutions was approximately pH 6.2. The collodion sacks remained in the culture solution for 24 hours, after which phosphorus was determined in the solution inside and outside of the collodion sack. The results are given in table 2, and show very clearly that when iron was present in the solution a considerable proportion of the phosphorus was non-diffusible. On the other hand, when iron was omitted from the solution all of the phosphorus was diffusible, as is indicated by the fact that the concentration of the solution inside the sack was the same as the concentration outside of the sack.

TABLE 2

Concentration of phosphate outside and inside of a collodion sack placed in the culture solution

IRON IN THE SOLUTION		IRON-FREE SOLUTION	
PO_4 outside sack	PO_4 inside sack	PO_4 outside sack	PO_4 inside sack
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
0.16	0.03	0.09	0.09
0.29	0.10	0.22	0.21
0.54	0.13	0.34	0.30
1.24	0.13	0.88	0.68
2.32	0.96	1.84	1.84
4.32	3.46	4.80	4.80
9.92	8.64	10.40	9.60

TABLE 3

*Amount of phosphate absorbed by corn from culture solutions with and without iron**

PO_4 IN SOLUTION		AMOUNT OF PO_4 ABSORBED	
		Iron in solution	Iron-free solution
p.p.m.	*mgm.*	*mgm.*	*mgm.*
0.05	0.37	0.05	0.18
0.10	0.75	0.04	0.39
0.25	1.87	0.37	0.97
0.50	3.75	0.75	2.25
1.00	7.50	0.90	5.78
2.00	15.00	6.60	10.42
5.00	37.50	7.50	8.63

* Absorption interval, 24 hours.
Age of plants, 19 days.

That this non-diffusible iron phosphate is not absorbed by the plant is shown by the data given in table 3. One series of plants was allowed to remain in the usual culture solution, while another series was placed in an iron-free culture solution. Both series were brought to the same phosphate concentration. After 24 hours, the concentration of phosphorus was determined in all cultures and the amount of phosphate absorbed was calculated. These data show that at the lower concentrations, phosphate absorption was much greater in the iron-free solution than in the regular culture solution containing iron. At concentrations of 2.0 and 5.0 p.p.m. PO_4 the presence of iron did not materially influence absorption, because there was insufficient iron to precipitate all of the phosphate.

After it was found that the presence of iron was preventing the absorptions of phosphate, iron was omitted from the culture solution. The plants were grown in the iron-free solution until they began to show a need for iron. When they became slightly chlorotic, iron was added to the solution for a period of one or two days. The solution was then changed, iron was omitted, and the plants were grown until they became chlorotic. It was necessary to add iron about once every week or ten days. More frequent additions were necessary in the early than in the late stages of growth.

That the modified procedure was more favorable for growth than the original procedure is shown by measurements of the plants. The corn in experiment 1 was grown for 2 weeks in the culture solution containing iron. At the end of the second week the iron-free solution was substituted for the regular solution. The height of the plants, measuring to the tip of the longest leaf, was determined at the end of the second and fourth weeks. Table 4 gives these measurements together with the increase in height for the 2 weeks following the change to the iron-free solution. It will be noticed that when 2 weeks old the plants in the cultures containing 1.0 p.p.m. PO_4 or more were decidedly better than the plants grown in solutions containing less than 1.0 p.p.m. PO_4. Following the change of solution, however, the plants grown in solutions containing 0.25 and 0.50 p.p.m. made good growth. The increase in height during the two weeks following the change of solution was very nearly the same for the cultures containing 0.25, 0.50, 1.0, and 2.0 p.p.m. PO_4.

TABLE 4

Height of corn 2 and 4 weeks after being placed in the culture solution

	CONCENTRATION OF PO_4 IN P.P.M.							
	0	0.05	0.10	0.25	0.50	1.00	2.00	5.00
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Height at 2 weeks	31.0	37.5	41.2	45.5	51.0	62.7	63.0	67.0
Height at 4 weeks	38.0	52.0	58.5	76.0	84.5	101.0	89.5	87.5
Increase last 2 weeks	7.0	14.5	17.3	30.5	33.5	38.5	26.5	20.5

The corn was harvested 36 days after planting, having been in the culture solution 31 days. The dry weight of roots and tops together with the percentage of phosphorus in each was determined and the data are recorded in table 5. Apparently maximum growth of corn was secured at a concentration of 1.0 p.p.m. PO_4. This agrees very well with the results of Hoagland and Martin (5). Rather satisfactory growth, however, was secured at concentrations of 0.50 and 0.25 p.p.m. PO_4. It is very probable that much better growth would have been secured at these concentrations if the iron-free culture solution had been used during the entire growing period. Absorption studies also indicated that any method of procedure that would tend more nearly to maintain the concentration of phosphate would undoubtedly favor better growth at the lower concentrations of phosphate.

In order to check the analytical work, both for adding phosphate and in the plant analysis, the amount of phosphate added during the growing period was compared with the amount recovered in the plant. These data are given in table 6. The last column of the table gives the percentage of added phosphate recovered in the plant. The percentage recovery was very good in all cultures except that containing only 0.05 p.p.m. PO_4 in solution. In both of the subsequent solution culture experiments the recovery of added phosphorus was very good—usually about 90 per cent.

TABLE 5

Dry weight and percentage phosphate in corn grown in solution cultures of the phosphate concentration indicated

PO_4 IN SOLUTION	DRY WEIGHT OF PLANTS			PO_4 CONTENT OF PLANTS	
	Tops	Roots	Total	Tops	Roots
p.p.m.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
None	1.15	0.95	2.10	0.25	0.28
0.05	2.30	1.35	3.65	0.28	0.28
0.10	3.45	1.85	5.30	0.30	0.37
0.25	6.90	3.20	10.10	0.46	0.56
0.50	10.60	4.05	14.65	0.46	0.55
1.00	18.00	6.00	24.00	0.67	0.77
2.00	16.85	7.45	24.30	0.94	1.08
5.00	19.77	6.05	25.82	1.17	1.34

TABLE 6

Amount of phosphate added to the culture solution, and the amount and percentage recovered in the plant

CONCENTRATION OF PO_4 IN CULTURE SOLUTION	PO_4 ADDED TO CULTURE SOLUTION	PO_4 RECOVERED IN PLANTS	ADDED PO_4 RECOVERED IN PLANTS
p.p.m.	*mgm.*	*mgm.*	*per cent*
0.05	5.89	4.53	76.8
0.10	13.01	11.66	89.5
0.25	48.84	48.96	100.0
0.50	75.24	65.01	86.4
1.00	162.30	161.51	99.5
2.00	246.83	233.74	94.7
5.00	298.33	306.74	102.6

The results of this experiment show that the maximum growth of corn may be secured in a solution containing 1.0 p.p.m. PO_4. The dialysis and absorption studies show that in solutions of low phosphate concentration it is necessary to omit iron from the solution except for periods of one or two days at 7- to 10-day intervals. Absorption studies similar to that given in table 3 show that the phosphate concentration was not maintained constant when two plants were grown per culture of 7500 cc. and the phosphate concentration was renewed twice a day.

Experiment 2—corn grown in culture solutions

Experiment 2 was planned to overcome some of the difficulties encountered in the first experiment. All cultures were in triplicate, with only one corn plant per culture of 7500 cc. An iron-free culture solution was used throughout the experiment except for an occasional period of one day, when the plants became somewhat chlorotic because of a deficiency of iron. The culture solution was analyzed for phosphate twice a day and the phosphate concentration renewed immediately after making the determinations. Even under this method of procedure the concentration of phosphate was not maintained satisfactorily. This is indicated by the absorption data of table 7.

TABLE 7

*Amount of phosphate absorbed, in 8- and 16-hour periods, from culture solutions of the phosphate content indicated**

PO_4 CONTENT OF SOLUTION		PO_4 CONTENT OF SOLUTION		PO_4 ABSORBED			
		After 8 hours	After 16 hours	In 8 hours		In 16 hours	
p.p.m.	*mgm.*	*p.p.m.*	*p.p.m.*	*mgm.*	*per cent*	*mgm.*	*per cent*
0.05	0.37	0.031	0.026	0.14	37.8	0.18	48.6
0.10	0.75	0.047	0.033	0.40	53.4	0.50	66.6
0.25	1.75	0.106	0.062	1.08	57.8	1.41	75.5
0.50	3.75	0.170	0.118	2.48	66.2	2.87	76.5
1.00	7.50	0.580	0.455	3.15	42.0	4.09	54.6

* The plants were 19 days old when the absorption study was started. There was one plant per culture of 7500 cc. All figures are the average of two or three cultures over a 4-day period.

The 8-hour period was from 8:30 a.m. to 4:30 p.m. The 16-hour period was from 4:30 p.m. to 8:30 a.m.

These data show that at all concentrations from 0.05 to 1.0 p.p.m. PO_4, the 19-day-old plants removed approximately one-half of the phosphate in the 8-hour period. During the 16-hour period from one-half to three-fourths of the phosphate was absorbed by the plant. At the lowest phosphate concentrations, 0.05 and 0.10 p.p.m. PO_4, almost as much phosphate was absorbed in 8 hours as was absorbed in the longer period. Apparently the plant could not readily reduce the concentration of phosphate lower than 0.03 p.p.m. PO_4. Using the data for the solution containing 0.10 p.p.m. PO_4, one can calculate the amount of phosphate that would be absorbed in 4 days if the phosphate concentration was renewed every 8 and every 16 hours—in the former case 4.8 mgm. of phosphate would be absorbed; in the latter case 3.0 mgm. would be absorbed. If the concentration could have been maintained at 0.10 p.p.m. it is probable that at least 6.0 mgm. PO_4 would have been absorbed and the growth would have been correspondingly better.

The corn of this series was harvested 33 days after planting. The dry weights of the plants are recorded in table 8 together with the percentage phosphate in the entire plants. All figures are the average of two or three cultures. In two or three instances one culture was omitted from the average because of insect injury.

These data show that maximum growth was secured at a phosphate concentration of 0.50 p.p.m. PO_4. The growth at 0.25 p.p.m. was very good and if this concentration of the solution could have been maintained it would doubtless have produced maximum growth. Growth at 0.10 p.p.m. PO_4 was approximately 40 per cent of the maximum but the absorption studies indicate that it would probably have been approximately 60 per cent of the maximum if the phosphate concentration could have been maintained constant. At the concentration of 0.05 p.p.m., growth was poor; under the most favorable conditions the growth would probably not have been one-half the maximum.

TABLE 8

Dry weight and phosphate content of corn grown in culture solutions of the phosphate concentration indicated

PO_4 IN SOLUTION	DRY WEIGHT OF PLANT			PO_4 IN ENTIRE PLANT	
	Top	Root	Total		
p.p.m.	*gm.*	*gm.*	*gm.*	*per cent*	*mgm.*
None	0.46	0.48	0.94	0.26	2.45
0.05	1.13	1.15	2.28	0.37	8.50
0.10	2.68	1.73	4.41	0.40	17.46
0.25	5.50	2.85	8.35	0.59	49.01
0.50	8.10	3.65	11.75	0.73	85.66
1.00	7.40	2.95	10.35	1.00	103.40
5.00	7.75	2.80	10.55	1.17	123.12

The percentage of phosphate in the plant increased with the phosphate concentration of the culture solutions, the percentage at the point of maximum growth, 0.50 p.p.m. PO_4, being twice as high as at the lowest concentration, 0.05 p.p.m. PO_4. These results are in close agreement with those of the first experiment. The percentage phosphate in the plant at the point of maximum growth was 0.69 and 0.73 per cent in experiments 1 and 2 respectively.

The data obtained in the second experiment seem to indicate that if one could maintain the phosphate concentration constant, maximum growth of corn under the conditions of this experiment would be obtained at a phosphate concentration of approximately 0.20 p.p.m. PO_4. It should be noted that the experimental conditions were favorable for very rapid growth and as a result the required rate of phosphate absorption was higher than usually obtains under field conditions. Experiment 2 was started July 1 and growth was very rapid. Four weeks after planting, the best plants had attained a height of 85 cm., measuring to the tip of the longest leaf. Under normal field conditions, it would probably require 6 weeks for the corn to make this growth. If the rate of growth is taken into consideration and if it is assumed that the soil maintained the phosphate concentration of the soil solution, it seems probable that good growth of corn would be secured if the soil solution contained 0.10 p.p.m. inorganic PO_4. It is very probable that a concentration of 0.15 or 0.20 p.p.m. would produce maximum growth under field conditions. The bearing of these results upon the question of the phosphate absorption by plants grown in soil will be considered later.

Experiment 3—corn grown in soil extracts

As a further means of studying growth at different concentrations of phosphate, corn was grown in the water extract of seven different soils. Soil was placed in collodion sacks and the plants were grown in the extract or diffusate. As the plant removed phosphorus from the extract more came into solution from the soil. The detailed procedure follows.

One hundred grams of dry soil was placed in each of twenty 500-cc. collodion sacks. Water was added to make a thin mud and the sacks were closed by tying with a soft cotton string. The 20 sacks were then placed in a 1-gallon earthenware jar and 3000 cc. of the regular nutrient solution was added to fill the jar. A glass tube entered the jar through a rubber stopper near the bottom. This provided a means of aeration and of stirring the extract, thereby hastening diffusion from the soil. Aeration was obtained by connecting all jars with an air pump which was operated by an automatic time switch. The switch was adjusted to operate the pump for a period of 3 minutes at intervals of 1 hour. The rate of bubbling in the jars was very rapid during the three minutes and seemed to afford very good aeration and stirring.

The phosphate content of the extracts was determined twice a week during the growing period. As the plants grew and absorbed considerable phosphorus the phosphorus content of the solution gradually decreased. The phosphate content indicated in table 9 is the average for the entire growing period.

The 7 soils used in the experiment were from plots whose phosphate treatments were known. All cultures were in duplicate with two plants to the culture. They were harvested 41 days after planting. Their dry weights and phosphorus content are given in table 9 together with the average content of inorganic and organic phosphate in the extracts. For convenience of comparison, the bottom line of the table gives the same data from the culture that produced maximum growth in the second experiment.

These data show, as the previous article points out, that growth is dependent on the presence of inorganic phosphorus. Where there was no inorganic phosphorus in solution there was little or no growth even though there was considerable organic phosphorus.

The growth obtained in the various cultures varied with the concentration of inorganic phosphate in solution. In general the data are in very close agreement with the data of experiment 2, given in table 8. The soil extract culture with an average phosphate content of 0.04 p.p.m. produced two plants weighing 7.22 gm. The solution culture of 0.05 p.p.m. produced 4.56 gm. (The weight of two plants is given instead of the weight of one, as is given in table 8.) The soil extract of 0.08 p.p.m. produced 6.45 gm. and the solution culture at 0.10 p.p.m. produced 8.82 gm. Finally, the soil culture with an average phosphate content of 0.19 p.p.m. produced 14.50 gm. Whereas the solution culture at a concentration of 0.25 p.p.m. produced 16.70 gm. In none of the soil cultures was maximum growth secured. This is indicated by comparing with the maximum growth of 23.50 gm. produced in the best cultures of experiment 2. It should be remembered that the growing period of experiment 2 was only 33 days whereas that of the soil extract cultures was 41 days.

TABLE 9

Dry weight and phosphorus content of corn grown in soil extracts of the phosphorus content indicated

SOIL	PHOSPHATE TREATMENT	PO_4 IN SOIL EXTRACT*		DRY WEIGHT OF TWO PLANTS	PO_4 CONTENT OF PLANTS	
		Inorganic	Organic			
		p.p.m.	*p.p.m.*	*gm.*	*per cent*	*mgm.*
Sandy loam	Acid phosphate	0.04	0.18	7.22	0.32	23.0
Sandy loam	None	Trace	0.16	2.22	0.31	6.9
Silt loam	Bone meal	0.08	0.18	6.45	0.35	22.5
Silt loam	Acid phosphate	0.19	0.21	14.50	0.34	48.8
Silt loam	None	0.02	0.14	3.97	0.29	11.3
Black clay loam	None	Trace	0.15	2.35	0.28	6.5
Silt loam	None	Trace	0.14	1.70	0.29	5.0
Culture solution	Experiment 2	0.50	None	23.50	0.73	171.32

* The figures are the average of 16 determinations made at regular intervals during the growing period.

The first culture of the soil extracts is of special interest in that it shows that fairly satisfactory growth can be secured at a rather low concentration of phosphate. During the first week the average phosphorus content of the extract was 0.12 p.p.m. inorganic PO_4. The plants made rather rapid growth, the phosphorus content fell to 0.04 p.p.m., and finally only a trace of inorganic phosphorus was in solution. As the phosphorus content decreased, the plants grew more slowly and at the time of harvesting they were making little or no growth. Apparently, the corn plant can absorb phosphorus from very dilute solutions but the rate of absorption is not sufficiently rapid to permit maximum growth.

Experiment 4—soybeans grown in culture solutions

It seemed advisable to study the growth of more than one crop at different concentrations of phosphate, accordingly soybeans were used in this experiment. In nearly all respects the method was the same as that used in the first experiment with corn. Laredo soybeans were germinated and two plants transferred to 7500-cc. culture vessels. As in experiment 1, the solution contained iron during the first two weeks but after that time an iron-free culture solution was used except for short intervals of time.

The soybeans made very satisfactory growth of tops but the root growth was not normal. There was very little branching of the roots and they never had the vigorous healthy appearance of the corn roots. This, however, probably did not seriously affect the results of the experiment. The soybeans were harvested 49 days after planting and the dry weight and phosphate content were determined as in previous experiments. These data are given in table 10.

TABLE 10

Dry weights and phosphate content of soybeans grown in culture solutions of the phosphate concentration indicated

PO_4 IN SOLUTION	DRY WEIGHTS OF PLANTS			PO_4 IN ENTIRE PLANT	
	Tops	Roots	Total		
p.p.m.	*gm.*	*gm.*	*gm.*	*per cent*	*mgm.*
None	0.60	0.17	0.77	0.27	2.08
0.05	1.52	0.40	1.92	0.28	5.45
0.10	2.10	0.47	2.57	0.31	7.99
0.25	6.12	0.75	6.87	0.37	25.49
0.50	10.32	1.20	11.52	0.51	59.10
1.00	10.20	1.52	11.72	0.73	85.44
2.00	11.80	1.10	12.90	0.92	118.03
5.00	11.20	1.15	12.35	1.06	130.91
10.00	10.25	1.17	11.42	1.22	139.32

A phosphate concentration of 0.50 p.p.m. PO_4 produced maximum growth of soybeans. Plants grown in the more concentrated phosphate solutions were more stocky and the stems were thicker than those of plants grown at 0.50 p.p.m. and less. With the lower phosphate concentrations the plants were more viney in growth. Although the maximum weight of plant was produced at 0.50 p.p.m., rather good growth was obtained at 0.25 p.p.m. There is no doubt that better growth would have been secured at the lower concentration if the experiment had been repeated and the procedure adopted in the second experiment with corn, used. The results obtained confirm those secured with corn and indicate that both crops require about the same concentration of phosphate for maximum growth.

DISCUSSION

The application of culture solution data to soil solution problems is not easy and is usually subject to different interpretations by different investigators. Hoagland (4) has pointed out many of the difficulties to be considered in applying culture solution data to soil solution problems. In spite of these difficulties, the culture solution method is useful, and provides a good means of studying various problems relating to the soil solution and plant nutrition. The culture solution data that are presented in this paper seem to have a very direct application to some soil solution problems.

Is the displaced solution adequate for plant growth?

The preceding experiments indicate that corn and soybeans will make maximum growth in a culture solution containing only 0.25 p.p.m. PO_4. As has already been stated, it is probable that maximum growth would be secured at even lower concentrations if the concentration of phosphate could be maintained constant and if the plants made a slower rate of growth as they normally do under field conditions. If those conditions are assumed it seems probable that good growth would be secured at a concentration of 0.10 p.p.m. PO_4 and maximum growth at 0.15 or 0.20 p.p.m. inorganic PO_4. These estimates appear to represent the very lowest concentrations that would possibly support good growth. None of the experimental results indicate that there is any probability that either corn or soybeans would make good growth in a culture solution containing much less than 0.1 p.p.m. PO_4. If 0.1 and 0.2 p.p.m. inorganic phosphate are the minimum concentrations for good and for maximum growth in culture solutions, what would probably be the minimum concentrations in the soil solution? Hoagland and Martin (5) have shown that absorption is more rapid from solution cultures than from sand cultures. The solid phase certainly would retard the movement and diffusion of salts thus tending to make it more difficult to maintain a given concentration at the absorbing surface of the root system. These considerations would seem to indicate that the minimum concentration for growth in the soil would probably be greater than in culture solutions. The soil, however, apparently can renew a given phosphorus concentration very readily. That being true, diffusion of phosphates may not be so important as in the case of some elements that are in solution but are not continually coming into solution from the soil. It seeems possible that the phosphorus concentration at the absorbing surfaces may be maintained rather constant by phosphorus coming into solution rather than by the diffusion of phosphorus from another portion of the soil solution. Granting that the phosphate concentration of the soil solution is maintained, there is no apparent reason for assuming that the plants would grow at a lower concentration than in culture solutions. Consequently, the figures given as a minimum for good growth in a culture solution would also be a minimum for good growth in the soil solution and there is a possibility that the minimum in the soil solution would be even higher than in the culture solution. The minimum concentration of phosphate essential for plant growth in the soil solution having been tentatively established, it is of interest to compare this result with the concentration of inorganic phosphorus in the soil solution obtained by displacement from productive soils.

In the accompanying article Pierre and the writer (14) have shown that the displaced solution from many productive soils may contain considerably less than 0.10 p.p.m. inorganic PO_4. Some of the displaced solutions contain less than 0.025 p.p.m. of inorganic phosphate, for example: The unfertilized plot of the Aledo, Illinois, experimental field has an average corn yield of 58.9 bushels. The displaced solution from this plot contained only 0.04 p.p.m. inorganic PO_4. Plot 2 of the Cullar's Rotation plots of the Alabama Experiment Station had an average corn yield of 43.2 bushels during the past four years. The plot has not received phosphate fertilization since 1911 but has been liberally fertilized with potassium and nitrogen. The displaced solution obtained from this plot contains only a slight trace of inorganic phosphorus. As has been shown, the organic phosphorus of soil extracts and soil solutions is not absorbed by plants. From these results it seems apparent that with some soils the phosphorus concentration of the displaced soil solution is not high enough to be adequate for plant growth. Presumably, therefore, in some soils there must be contact between the plant root and the solid phase before the plant can absorb the phosphorus necessary for growth.

These considerations probably are not applicable to the absorption of any other of the essential elements, with the possible exception of iron. We have obtained some experimental results indicating that the solution displaced from limed soils does not contain iron in a concentration sufficient to prevent chlorosis in young plants. Plants grown in these soils have a normal color and show no evidence of a deficiency of iron. The results obtained with iron confirm the results of the phosphate studies in that they indicate that contact between root and soil is necessary for the absorption of iron from soils.

The absorption of phosphorus from the soil

If plants do not grow in the soil solution obtained by displacement, we may explain growth and the absorption of phosphorus and iron by one or more of the following assumptions:

1. The displaced solution is not the true soil solution and consequently the experimental method is erroneous.
2. The plant roots exert a solvent action on the soil particles and thus bring more phosphorus into solution, producing a higher phosphate concentration at the absorbing surfaces.
3. As a result of a Donnan equilibrium, there is a higher concentration of phosphorus in the solution at the surface of the soil particles than in the displaced soil solution. The plant roots, being in very intimate contact with the soil particles, absorb most of their phosphorus from this more concentrated solution.

Each of these assumptions will be considered in some detail.

As far as all experimental evidence has shown, there can be little doubt but that the displacement method gives a solution identical with or at least very similar to the true soil solution. The work of Parker (8), and subsequently that of Burd and Martin (1), clearly established the value of the method and proved that it apparently gives the true soil solution. In a more recent paper Burd and Martin (3) have briefly summarized the experimental evidence tending to prove that the true soil solution is obtained by displacement. The experimental facts tending to prove that the method gives the true soil solution, do not necessarily prove that there is not a more concentrated layer of solution at the surface of the particle as a result of a Donnan equilibrium. If it should be proved that such a layer does exist at the surface of the solid phase the same data would prove that the displacement method does not give the true soil solution in the strict meaning of that term.. Until such evidence is advanced it seems correct to assume that the soil solution is obtained by displacement. That being the case, the absorption of phosphorus from some soils must be explained by one or both of the other possible assumptions that have been given.

The theory of the solvent action of plant roots is very old and there is considerable experimental evidence bearing on the question. The etching action of plant roots on marble plates is easily demonstrated. Kossovitch (6) and Marias (7) have shown that plants do not utilize insoluble phosphates very readily unless there is contact between the roots and the phosphate particles. However, there apparently is very little evidence to show that the solvent action of the roots could be due to anything but the carbon dioxide that is given off as a result of plant respiration. The writer (9, 10) has reported some experiments which were planned to determine whether the carbon dioxide production of plant roots was a factor in the "feeding power" of plants for inorganic elements. None of the several experiments reported indicated that the carbon dioxide production of the roots was an important factor in the absorption of inorganic elements by any crop. These experiments, however, may not be absolutely conclusive. As was pointed out in the first paper (9, p. 238) the root surfaces are in such intimate contact with the soil particles that the solvent action of the carbon dioxide might take place to some extent before it could be removed by rapid aspiration. It seems that it would be impossible experimentally to prevent the possibility of any solvent action of the roots.

In order to obtain further evidence bearing on the carbon dioxide theory some tests were made to determine whether carbon dioxide had a very marked solvent action on soil phosphorus. In the first tests a stream of carbon dioxide was passed through a jar of moist soil for a period of 24 hours. The soil was then packed in the displacement cylinders. While being packed, the displacement cylinders were repeatedly filled with the gas, thus maintaining a very high concentration of carbon dioxide in the soil solution. The results of these tests are given in table 11 and show that the carbon dioxide treatment did not materially influence the inorganic phosphorus content of the displaced solution. On the other hand, the carbon dioxide treatment practically doubled the calcium content of the solution from the sandy loam soil. This increased amount of calcium apparently caused the precipitation of some of the phosphorus.

The solvent action of carbon dioxide was next studied by preparing water extracts with carbon-dioxide-free water and water saturated with carbon dioxide. The extracts were prepared by the collodion sack method (13). When the carbon-dioxide-saturated water was shaken with the soil some of the gas was given off producing considerable pressure in the flasks. The treatment, therefore, may be considered rather drastic. The results obtained with five soils are given in table 12.

TABLE 11

The phosphorus and calcium content of the displaced solution from untreated soil and soil treated with carbon dioxide

SOIL	CONCENTRATION IN DISPLACED SOLUTION					
	Inorganic PO_4		Organic PO_4		Calcium	
	Untreated	CO_2	Untreated	CO_2	Untreated	CO_2
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Sandy loam	0.06	0.03	0.20	0.27	25.4	48.4
Silt loam	0.03	0.04	0.27	0.16	339.0	360.0

TABLE 12

The concentration of phosphorus, calcium and potassium in soil extracts prepared with distilled water and with water saturated with carbon dioxide

SOIL	CONCENTRATION IN SOIL EXTRACT							
	Inorganic PO_4		Organic PO_4		K		Ca	
	H_2O	CO_2	H_2O	CO_2	H_2O	CO_2	H_2O	CO_2
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Sandy loam	0.03	0.10	0.17	0.08	0.68	0.99	1.9	10.6
Clay loam	0.05	0.10	0.11	0.12	0.31	0.37	4.8	14.1
Silt loam	Trace	0.02	0.05	0.10	0.34	0.44	14.2	31.0
Sandy loam	Trace	0.04	0.11	0.08	3.12	3.75	4.4	7.0
Silt loam	Trace	Trace	0.06	0.10	0.99	1.64	6.1	16.2

The carbon dioxide treatment caused an appreciable increase in the inorganic phosphorus content of the extract from the soils. The concentration, even after treatment, was very low considering the fact that several of the soils are very productive. When the drastic treatment given is considered, the experiment does not indicate that the carbon dioxide given off by the plant roots would have much of a solvent action on soil phosphorus. The solvent effect of the carbon dioxide, as would be expected, was greater with calcium than with either phosphorus or potassium. When these results and the experimental work previously mentioned are considered, it seems that little significance should be attached to the theory until new experimental evidence is advanced in its support.

Comber (3) has emphasized the importance of root-soil contact for the absorption of phosphorus and iron by the plant. Although emphasizing the importance of root-soil contact, he does not attribute very much importance to the production of carbon dioxide by the roots. He suggests two hypotheses: first, the absorption of colloids; and second, the solvent action of various organic substances of the plant sap. The second hypothesis seems, to the writer, more acceptable than the first. The contact of root hair and soil colloid is considered to be so close that one system is formed and that there is the possibility of the cell sap having a solvent action on soil colloids without the excretion of acid into the soil generally. The nature of the root-soil contact is generally recognized by plant physiologists, and the solubility effects mentioned by Comber may be studied in the laboratory. In addition to the solubility effects, the enzymes of the plant roots may decompose some of the organic matter of the soil. This may be of special importance with reference to phosphorus absorption, since the preceding paper shows that most soils contain considerable organic phosphorus in solution. The root-soil colloid system would probably permit of greater enzymatic activity than would culture solutions.

As a result of earlier work on the concentration of phosphorus in the soil solution the suggestion was advanced that a Donnan equilibrium produced a higher concentration of phosphorus at the surface of the solid phase than in the balance of the soil solution which is represented by the displaced solution. The data presented in this paper afford indirect evidence supporting that hypothesis in that they show that plants do not make good growth at as low a concentration of inorganic phosphorus as is found in the displaced solution of many productive soils. Apparently, the plants must absorb their phosphorus from a more concentrated solution, such as that assumed by the hypothesis.

It is difficult to secure direct experimental proof as to whether there is a Donnan equilibrium in soils, although several experiments have been made in an effort to secure such proof. Some of the results seem satisfactory but they usually can be explained by assuming that the roots exert a solvent action on the soil particles. The work is being continued, but at present there is little conclusive evidence as to which of the two assumptions is more nearly correct. Possibly both are operating and there may be others of some importance that have not been considered.

SUMMARY

Four experiments are reported in which a study was made of the concentration of phosphate necessary for the maximum growth of corn and soybeans. The experimental procedure was described in detail, special attention being given to the method of renewing the phosphate concentration in an effort to maintain it as constant as possible. The rate of absorption of phosphate from solutions of different concentration was studied and the results are presented in table 7. The bearing of the experimental data on present theories of plant nutrition was discussed in some detail. The results of the investigation may be briefly summarized as follows.

1. In order to provide favorable conditions for plant growth in culture solutions of low concentrations of phosphate, it is necessary to use a large volume of solution for each plant, to renew the phosphate concentration at very frequent intervals, and to grow the plants in an iron-free culture solution. Iron must be supplied occasionally for a period of one to two days
2. The maximum growth of corn was secured in a solution containing 0.50 p.p.m. PO_4. Growth at 0.25 p.p.m. was good, the dry weight being about 75 per cent of the maximum.
3. If it is assumed that the concentration of phosphate could be constantly maintained and that the rate of growth was more nearly comparable to field conditions it seems probable that the maximum growth of corn would be secured at a phosphate concentration of 0.15 or 0.20 p.p.m. PO_4. Fair to good growth might be secured at a concentration of 0.10 p.p.m. PO_4.
4. Maximum growth of soybeans was secured at a phosphorus concentration of 0.50 p.p.m. PO_4. Apparently there is little or no difference between corn and soybeans as regards the phosphate concentration necessary for good growth.
5. Since the displaced solutions of many productive soils contain only a trace of-inorganic phosphorus it seems necessary to assume that plants do not obtain all of their phosphorus from the solution represented by that obtained by the displacement method. Apparently the solid phase of the soil has an important function in the phosphorus nutrition of the plants
6. The phosphorus nutrition of plants in soils with solutions of low phosphate concentration may be explained by the assumption that the plant roots have a solvent action on the solid phase. As a result of the assumed solvent action, the phosphate concentration at the absorbing surface would be greater than in the solution obtained by displacement. Another possible explanation for the phosphorus nutrition of plants is that a Donnan equilibrium is found at the surface of the solid phase. As a result, the solution at this point would have a higher phosphate concentration than that of the free soil solution represented by the displaced solution.
7. Most of the available experimental data seem to indicate that the theory of the solvent action of carbon dioxide from plant roots does not adequately explain the absorption of phosphorus from many soils.
8. At present there is no definite proof that a Donnan equilibrium exists in soils, as has been suggested. Granting that it does exist there is no proof as to what is the concentration of the solution at the surface of the soil particles.

REFERENCES

(1) BURD, J. S., AND MARTIN, J. C. 1923 Water displacement of soils and the soil solution. *Jour. Agr. Sci.* 13: 265-295.
(2) BURD, J. S., AND MARTIN, J. C. 1924 Secular and seasonal changes in the soil solution. *Soil Sci.* 18: 151-167.
(3) COMBER N. M. 1922 The availability of mineral plant food. *Jour. Agr. Sci.* 12: 363–369.
(4) HOAGLAND, D. R. 1925 Physiological aspects of soil solution investigations. *Hilgardia* 1: 227–257.
(5) HOAGLAND, D. R., AND MARTIN, J. C. 1923 A comparison of sand and solution cultures with soils as a media for plant growth. *Soil Sci.* 16: 367–388.

(6) KOSSOVITCH, P. S. 1902 The rôle of the plant in dissolving the plant food of the soil. *Zhur. Opytn. Agron.* (*Russian*) 3 (2): 145–180.

(7) MARIAS, J. S. 1922 The comparative agricultural value of insoluble mineral phosphates of aluminum, iron, and calcium. *Soil Sci.* 13: 355–409.

(8) PARKER, F. W. 1921 Methods of studying the concentration and composition of the soil solution. *Soil Sci.* 12: 209–232.

(9) PARKER, F. W. 1924 Carbon dioxide production of plant roots as a factor in the feeding power of plants. *Soil Sci.* 17: 229–247.

(10) PARKER, F. W. 1925 The carbon dioxide content of the soil air as a factor in the absorption of inorganic elements by plants. *Soil Sci.* 21: 39–44.

(11) PARKER, F. W., AND FUDGE, J. F. 1927 Soil phosphorus studies: I. The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil Sci.* 24: 109–117.

12) PARKER, F. W., AND TIDMORE, J. W. 1926 The influence of lime and phosphatic fertilizers on the phosphorus content of the soil solution and of soil extracts. *Soil Sci.* 21: 425–442.

(13) PIERRE, W. H., AND PARKER, F. W. 1927 The use of collodion sacks in obtaining clear soil extracts for the determination of the water-soluble constituents of soils. *Soil Sci.* 23: 13–32.

(14) PIERRE, W. H., AND PARKER, F. W. 1927 Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and the availability of organic phosphorus to plants. *Soil Sci.* 24: 119–128.

THE DELAYED EFFECT OF LIMING

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Price has reported that yields of alfalfa hay were much greater when the crop was seeded in the year following the limestone application than when seeded in the same year (2). Possibly it is a matter of rather frequent observation that for some time after application limestone is not so effective as it is later.

The delayed effect of limestone in bringing about increased growth of alfalfa was shown in a marked way in experiments reported in a previous paper (1).

In addition to the pots containing only field acid soil and only field limed soil, results from which were given in the previous paper, pots were included containing the field acid soil limed just before potting. The lime treatment consisted of thoroughly mixing 5 gm. of limestone ground through a 40-mesh sieve, with the 2000 gm. of air-dry soil contained in a pot.

In the case of the first experiment with the porous earthenware pots, where the Berea and Mayfield experiment field soils were used, the Berea acid soil limes just before potting gave practically no better growth of alfalfa during the entire eight weeks of the experiment than did the untreated acid soil. The Mayfield acid soil limed just before potting gave no better growth than did the untreated acid soil for the first four weeks of the experiment. Growth then began and was fairly satisfactory during the remaining four weeks of the experiment.

The air-dry weights of tops and roots from the pots containing the field acid and field limed soils and the field acid soil limed just before potting are given in table 1.

These pots were allowed to remain in air-dry condition from the date the alfalfa growth was removed (July 1) to December 12 of the same year. Without further treatment they were again planted to alfalfa. This second growth of alfalfa was as good on the acid soils limed just before plotting (pots 9, 19) as on the field limed soils (pots 8, 18).

In the second experiment with the porous earthenware pots, where the Mayfield, Berea, and Greenville experiment field soils were used, growth on the pots containing acid soil limed just before potting was, on the whole, but little better than on the untreated acid soils for the first 8 weeks of the experiment.

This experiment included pots containing field limed soil that had received, just before potting, the same liming treatment as the acid soil—5 gm. limestone ground through a 40-mesh sieve, to every 2000 gm. of soil in a pot. On the lime-treated pots increasing growth of alfalfa over that on the soil with only the field lime treatment was evident from about six weeks after germination of the plants, and while there was still no effect of the treatment applied to the acid soil as compared with the acid soil untreated. Evidently in these experiments the delayed effect of limestone in increasing plant growth is due to the slowness with which the beneficial changes in the soil come about and not to what might be a possibility, that some temporary objectionable condition results from liming which for a time offsets its beneficial effect.

Knowledge of the delayed effect of liming is important in a practical way in indicating the desirability of liming some time, several weeks or preferably longer, before seeding an acid-sensitive legume crop. It is also important in pot work of short duration where liming treatments are included, since only a small part of the expected effect of liming is likely to be secured under such conditions. It was particularly in view of the bearing on pot experiments that it was thought of interest to call attention to the results given in this paper.

TABLE 1

Air-dry weights of the tops and roots from the field acid and field limed soils and from the field acid soil limed just before potting

POT NUMBER		NATURE OF SOIL	MAYFIELD SOIL—AIR-DRY WEIGHT OF		BEREA SOIL—AIR-DRY WEIGHT OF	
Mayfield soil	Berea soil		Tops	Roots	Tops	Roots
			gm.	*gm.*	*gm.*	*gm.*
7	17	Acid	0.403	0.139	0.053	0.017
8	18	Field limed soil	2.260	0.796	2.094	0.790
9	19	Acid, but limestone added just before potting	1.162	0.208	0.034	0.013

SUMMARY

A delayed effect of limestone in bringing about increased growth of alfalfa on acid soils in pot experiments was noted. The interval ranged from a few weeks to about two months, depending on the soil.

Knowledge of this delayed effect is important in indicating the desirability of liming some time ahead of seeding legume crops. It also has an obvious bearing on pot experiments of short duration where liming treatments are given.

REFERENCES

(1) Karraker, P. E. 1927 Production of nodules on different parts of the root systems of alfalfa plants growing in soils of different reaction. *Soil Sci.* 24: 103-107.

(2) Price, O. B. 1923 Fertilizers affect quality of alfalfa. *Mich. Quart. Bul. v.* 5: 158-159.

THE EFFECT OF PROTOZOA AND FUNGI ON CERTAIN BIOCHEMICAL PROCESSES WHEN INOCULATED INTO PARTIALLY STERILIZED SOIL[1]

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That treatment of soil by heat or by volatile antiseptics causes an increase in the yield of the following crop has been known for nearly half a century. Hiltner and Störmer (14) in 1903, and later Russell and Hutchinson (33, 34) and others showed that such treatment also causes a temporary decrease in numbers of bacteria as determined by the plate method, usually followed by an increase to numbers much in excess of those initially present in the soil. Coincident with this increase of bacteria, there is a large accumulation of ammoniacal nitrogen. This is partially explained by the fact that the nitrifying bacteria are killed and the ammonia thus accumulates instead of being oxidized to nitrates as in normal soils. Nevertheless, the ammoniacal nitrogen thus accumulating is very much in excess of the nitrates ordinarily found. When soil thus "partially sterilized," in the terminology of Russell and Hutchinson, is reinoculated with a suspension of fresh soil, the ammonia is rapidly oxidized to nitrates and the bacterial numbers eventually fall below those in partially sterilized soils not reinoculated.

The explanation for these and many other phenomena of partial sterilization is very obscure. Many theories (42) have been advanced, the most noted of which is the protozoan theory of Russell and Hutchinson (33, 34). According to this theory, there is a factor in soils which limits the bacterial action. This limiting factor is the protozoa, which are known to feed on bacteria, and the active cells of which are killed by partial sterilization. Hence, when the soil is partially sterilized, the few surviving bacteria are able to multiply and thus reach higher numbers than they would if the protozoa were present and active. This explains why inoculation of a partially sterilized soil with a soil suspension containing, of course, protozoa causes the diminution of the numbers of bacteria.

This theory has been attacked from various angles. It was maintained, particularly in the United States, that protozoa were normally present in the soil largely, if not exclusively, as cysts, and thus were in an inactive state (2, 15, 36, 28, 16). Martin and Lewin (25, 26) and Goodey (10), however, recognized a trophic fauna in English soils, as did also Waksman in American soils (39, 40). The recent work of Cutler and associates (3, 5, 7, 8), Perey (30), Allison (1), and Sandon (35), has demonstrated conclusively that at least amoebae and flagellates are normally found in a trophic condition in soils from England, France, and the United States. Recently Koffman (17) has examined a large number of Swedish soils by a direct method and has found a trophic fauna of amoebae, and more particularly of small flagellates. He has never found trophic ciliates.

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Likewise, the contention that protozoa limit the numbers of bacteria in soil has not received unanimous assent. Russell and Hutchinson (34) were not always able to reduce the number of bacteria by additions of mass cultures of protozoa, nor could Greig-Smith (11), Goodey (10), or Sherman (36) usually get a reduction in numbers of bacteria by reinoculation with protozoa cultures. Cunningham (2) and Waksman (39, 40), found that protozoa seemed to limit bacterial numbers. It must be confessed that none of these results is very convincing. As pointed out by Sherman, in freeing crude bacterial cultures from protozoa, the resulting cultures would differ from the crude ones. Many of the bacterial species would undoubtedly be lost and, in addition, because of selective feeding on the part of the protozoa, the ratios of one species to another might differ. Then too, in nearly all this work, there has been no assurance that the protozoa actually excysted and multiplied. Controlled inoculation work by Cutler (4) wherein the course of the activity of the protozoa was carefully followed by adequate technique, has demonstrated that the numbers of bacteria in sterilized and reinoculated soils are kept down by certain protozoa. Unpublished work of Cutler and Dixon (6) confirms the earlier work of Cutler. In daily examinations of soil through a period of one year, whereby both the bacteria and the protozoa were counted, it was also found by Cutler, Crump, and Sandon (7) in 86 per cent of the observations that an inverse relationship existed in a Rothamsted soil between bacteria, as determined by the plate count, and certain active amoebae.

That protozoa exist in the soil in a trophic state, and that they limit to some extent the numbers of bacteria is now fairly well established. Whether they limit or influence in any way the various microbiological activities, however, is another matter. Very little work has been done on this subject. Hills (13) studied the accumulation of available nitrogen (ammoniacal and nitrate) from sterilized soils which were reinoculated with soil known to contain protozoa on one hand and with a crude culture of bacteria obtained by picking several colonies from agar plates poured from soil dilutions on the other. He obtained no evidence of decreased accumulation of available nitrogen caused by protozoa. Cutler (32) criticized the technique, and the criticism is equally valid against the work of Cunningham (2) who obtained results which would tend to substantiate the protozoan theory. Lipman, Blair, Owen, and McLean (24) studied the accumulation of ammonia in soils to which organic nitrogenous matter such as dried blood or soybean meal had been added. They partially sterilized these soils, to some of which they afterwards added an inoculum of untreated soil, and to the others an inoculum of a soil freed from protozoa by partially sterilizing it. They obtained no results which showed a depressing action by protozoa. There is no certainty here, however, that the protozoa excysted and multiplied. Waksman (39, 40) found that protozoa caused no diminution of NH_3 formation, but unfortunately he did not include in his studies the amoebae, which are now thought to be of very much more importance than the other soil protozoa. Nasir (29) has shown that in culture media certain protozoa caused an increase in nitrogen fixation by *Azotobacter*. It would appear that the question whether protozoa markedly influence biochemical transformations in the soil is still an open one.

In all this work, it must be noted, from the classical work of Russell and Hutchinson onward, only bacteria and protozoa were considered. The fungi, algae, and actinomycetes were almost entirely ignored. This is unfortunate, for it is now known that the fungi, at least, are very important. Thus, when soil is partially sterilized, the fungi are either entirely killed or their action is arrested for a time. If the soil is reinoculated on the one hand with a soil suspension and on the other with crude cultures of bacteria or a suspension of partially sterilized soil, a very important factor has been introduced in the one case but not in the other. The other members of the soil population, of whose action we know so little, are also introduced with an untreated soil suspension.

Recently, a detailed and important contribution by Waksman and Starkey (42) which concerns itself largely with this phase of the subject has appeared. Unfortunately these workers did not avail themselves of the Rothamsted improvements in soil protozoological technique and the small amount of work done by them on protozoa is of limited value.

All of the experimental work with soil protozoa until recently has dealt with crude cultures. These contained one or more protozoan species growing in cultures of bacteria of unknown numbers and species, and might or might not contain fungus spores. Accurate reinoculation work is needed whereby one species of protozoa with a definite number of one species of bacteria is added to a sterlized or partially sterilized soil, and this contrasted with a soil similarly treated and reinoculated with the same number of that particular bacterial species free from protozoa. This work has not heretofore been done in soil, except in relation to numbers of bacteria by Cutler (4). It was to study the effect of protozoa on soil activities by such technique that the present work was undertaken.[3]

EXPERIMENTAL

Moist soil from Barnfield plot 4, A, which has been fertilized for 69 years with complete minerals and ammonium sulphate, and which is still basic, was used in the first experiment. It was passed through a 3-mm. sieve and 200 gm. placed in each of twelve 300 cc. Erlenmeyer flasks. Ten of these flasks were placed in cold water and slowly brought to 80°C., and were kept at 80 to 85° for 1 hour. This was sufficient to eliminate all the protozoa and fungi. After being cooled for 24 hours they were reinoculated in various ways as shown in table 1.

TABLE 1

Treatment of the soil used in experiments

FLASK NUMBER	TREATMENT
1, 2	Untreated soil, 2 cc. sterile soil infusion added
3, 4	Partially sterilized soil, 2 cc. sterile soil infusion added
5, 6	Partially sterilized soil, 2 cc. protozoa culture added
7, 8	Partially sterilized soil, 2 cc. bacterial culture added
9, 10	Partially sterilized soil, 2 cc. fungus culture added
11, 12	Partially sterilized soil, 2 cc. fresh soil infusion added

The protozoa culture consisted of 12,400 cysts of *Hartmanella hyalina*—one of the commonest of soil amoebae—growing in a culture with 9,000,000 bacterial vegetative cells of a small spore-forming rod. Both bacterial and protozoan numbers were determined by the haemocytometer method. The bacterial inoculum consisted of 9,000,000 vegetative cells of the same bacterial species free from protozoa, in the same medium, which was a sterile soil infusion. The fungus inoculum was a suspension in sterile soil infusion of spores of *Trichoderma köningi* and a green *Penicellium* of the *glaucum* type.

The soil infusion was from the original Barnfield plot. The moisture was approximately three-fourths saturation, full saturation being 37.2 per cent on the dry soil basis. A respiration apparatus similar to that used by Waksman and Starkey (43) was used to determine the CO_2 given off. The standard oxalic acid and barium hydroxide was made so that 1 cc. equalled approximately 0.5 mgm. of carbon as CO_2. The first day's accumulation of CO_2 was not measured.

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[3] The writer wishes to thank the authorities of the International Education Board and of the New Jersey Agricultural Experiment Station, who made it possible to carry out these studies at Rothamsted. The work was done under the direction of D. Ward Cutler, M.A., of the Department of General Microbiology, to whom the writer desires to express sincere appreciation for ever-ready help and advice. Also very grateful thanks are extended to Sir John Russell for his hospitality in allowing the writer to use the laboratories at Rothamsted.

At the end of 14 days the experiment was discontinued because of an accident, and the bacteria and protozoa were counted. The bacteria were estimated by the common plate method, five plates of Thornton's (38) medium being used for each dilution, and the incubation time being 10 days at about 20°. The protozoa were determined by the method in use at this laboratory (7). The results are given in tables 2 and 3.

TABLE 2

The effect of different reinoculation treatments on the CO_2 evolution from partially sterilized soil

Milligrams C as CO_2

DAYS	CHECK		NO REINOCULATION		REINOCULATED WITH PROTOZOA		REINOCULATED WITH BACTERIA		REINOCULATED WITH FUNGI		REINOCULATED WITH SOIL	
	1	2	3	4	5	6	7	8	9	10	11	12
2	1.4	1.3	1.2	2.1	1.8	1.6	1.7	1.6	1.9	1.5	1.3	1.2
3	1.9	1.9	2.2	1.8	2.3	1.9	1.6	1.8	2.2	2.2	2.2	1.0
3	2.3	2.1	2.8	2.8	2.8	2.6	4.1	3.9	3.4	4.1	4.0	4.2
3	2.3	2.2	2.8	2.6	3.4	3.1	3.8	3.6	3.3	4.0	3.4	4.3
3	3.4	3.4	4.5	3.8	3.6	3.7	3.8	4.4	4.7	4.9	4.3	3.9
Total 14	11.3	10.9	13.5	13.1	13.9	13.1	15.0	15.3	15.5	16.7	15.2	14.6

TABLE 3

The effect of re-inoculation of partially sterilized soil with bacteria and with protozoa

Incubation 14 days

NUMBER	TREATMENT	BACTERIA PER GRAM*
		millions
1	Control. No treatment	32.6 } ave. 30.2
2	Control. No treatment	27.8
5	Reinoculated with *Hartmanella*	145.5 } 157.5
6	Reinoculated with *Hartmanella*	169.6
	Reinoculated with bacteria	208.4 } 204.2
	Reinoculated with bacteria	200.0

* Moist soil basis.

The CO_2 results are inconclusive but they seem to indicate that the CO_2 may possibly be depressed by the protozoa; however, it is of great interest to note that the addition of fungi to partially sterilized soil resulted in a greater increase in the evolution of CO_2 than even the inoculation with the bacteria.

The results of bacterial counts presented in table 3 confirm the results of Cutler (4) that some protozoa, such as *Hartmanella*, are capable of reducing to a certain extent the numbers of bacteria in the soil. Trophic amoebae were found in the soils of flasks 1, 2, 5, 6, 11, and 12.

The experiment was repeated a second time and run for 69 days. This time 4,600 *Hartmanella* cysts were inoculated with 50,000 bacterial cells of a small non spore-forming yellow rod. This was found to be a very good bacterium on which to grow *Hartmanella*. The soil was from the heavily manured Barnfield plot 1, 0. At the end of the experiment the ammoniacal and nitrate nitrogen was determined. The NH_3 nitrogen was determined by the Harper (12) method. After distillation with MgO, 10 cc. of 2*N* NaOH and 200 cc. of H_2O were added and 200 cc. was boiled off. More water and 2 gm. of Devarda's alloy were then added and the nitrates distilled off as NH_3. Ammonia-free water was used throughout. Special tin-lined stills and for titration only 0.02 *N* standard acid and alkali were used. Methyl red was the indicator employed and the excess of CO_2 was boiled off when the solution was still slightly acid. Very small differences in nitrogen could thus be measured accurately. The results are given in tables 4 and 5. The fungi were counted by the method suggested by Waksman and Fred (41). Trophic amoebae were found only in flasks 2, 5, 6, 11, and 12, although cysts of *Naegleria gruberi* were found in flask 1.

TABLE 4

The effect of different re-inoculation treatments on the CO_2 evolution from partially sterilized soils

Milligrams C as CO_2

DAYS	CHECK		NO REINOCULATION		REINOCULATED WITH PROTOZOA		REINOCULATED WITH BACTERIA		REINOCULATED WITH FUNGI		REINOCULATED WITH SOIL	
	1	2	3	4	5	6	7	8	9	10	11	12
6	4.0	3.8	10.9	10.1	8.2	8.9	8.7	10.1	9.1	9.3	8.8	9.4
7	6.7	6.5	7.7	7.4	7.2	7.2	8.0	8.1	9.0	9.4	9.6	9.9
6	3.0	2.8	4.0	4.2	4.2	3.6	4.1	5.2	5.0	4.6	4.8	3.2
7	2.0	2.2	4.8	4.0	3.0	1.9	3.4	3.1	3.8	4.8	3.2	3.6
6	2.6	2.2	3.1	3.0	3.4	1.0	3.8	4.2	5.6	5.5	1.0	3.7
7	1.5	1.6	3.5	3.8	5.8	5.5	4.4	5.3	5.8	6.0	1.3	3.3
9	2.9	3.6	5.0	4.3	3.3	2.2	3.4	3.2	6.0	3.0	0.9	4.4
9	4.6	4.4	4.8	4.3	3.9	3.2	4.0	4.0	5.0	4.6	2.8	4.2
12	5.6	6.2	5.6	5.4	4.6	4.2	4.8	4.8	6.0	7.5	4.2	6.6
Total 69	32.9	33.3	49.4	46.5	43.6	37.7	44.6	48.6	55.3	54.7	36.6	48.2

A third experiment like the above except that only the available nitrogen was determined, was run for a period of 115 days. The flasks were not aerated but had cotton stoppers. Protozoa were found in the soil of flasks 5 and 6 but the numbers were not determined. The results are shown in table 6.

A study of tables 2, 4, 5, and 6 shows that whereas the protozoa seem to have depressed both the CO_2 evolution and the available nitrogen accumulation, the fungi stimulated the CO_2 evolution and depressed the nitrogen accumulation to a greater extent. The effects of the fungi were beyond the limits of experimental error. The protozoa, however, have had an effect that is not so marked, and may even be doubted from the data so far presented. It will be seen that the variations between duplicate flasks are not large, except in the case of the untreated soil and the soil inoculated with a soil suspension. This is to be expected since there is a heterogeneous flora and fauna,

TABLE 5

The effect of partial sterilization of soil and subsequent reinoculation with various agents on the available nitrogen, and microbiological flora

Incubation 69 days

FLASK NUMBER	TREATMENT	NH_3 N	NO_3 N	TOTAL AVAILABLE N†	BACTERIA PER GRAM‡	FUNGI PER GRAM‡
		p.p.m.	*p.p.m.*	*p.p.m.*	*millions*	*thousands*
1	Untreated soil	9.1	19.1	28.2 ave. 30.7	46.2	380
2	Untreated soil	12.5	20.7	33.2	47.0	316
3	Partially sterilized soil	71.6	14.1	85.7 86.6	56.0	0
4	Partially sterilized soil	68.2	19.4	87.6	57.0	0
5	Partially sterilized soil, reinoculated with protozoa	64.7 63.9 ave. 64.3	9.1 9.1 ave. 9.1	73.4 71.5	122.4	0
6	Partially sterilized soil, reinoculated with protozoa	61.4 60.6 61.0	8.3 9.1 8.7	69.7	163.4	0
7	Partially sterilized soil, reinoculated with bacteria	65.5 65.5 65.5	11.6 10.8 11.2	76.7 77.1	171.2	0
8	Partially sterilized soil, reinoculated with bacteria	65.5 64.7 65.1	12.4 12.4 12.4	77.5	214.2	0
9	Partially sterilized soil, reinoculated with fungi	42.3	12.4	54.7 56.7	92.0*	§
10	Partially sterilized soil, reinoculated with fungi	43.1	15.7	58.8	52.0*	§
11	Partially sterilized soil, reinoculated with soil infusion	5.8	73.0	78.8 77.6	62.8	2400
12	Partially sterilized soil, reinoculated with soil infusion	24.2	52.3	76.5	......	1540

* Fungi interfered with accurate counting of bacteria.

† Dry soil basis.

‡ Moist soil basis.

§ Too many to count.

making variations in growth inevitable, whereas where pure cultures are added, more uniform results are to be expected.

To make certain that the differences between the flasks with and without *Hartmanella* were not due to chance another experiment was set up—6 flasks like numbers 5 and 6 of the above experiment with protozoa, and 6 like numbers 7 and 8 without protozoa. This time 12,400 cysts and 50,000 bacterial cells were added. The results are given in table 7.[4] The available nitrogen was determined as before, after longer incubation, and the results are given in table 8.

TABLE 6

The effect of reinoculation of partially sterilized soil with various agents, upon the accumulation of available nitrogen

Incubation 115 days

FLASK NUMBER	TREATMENT	NH_3		NO_3		TOTAL AVAILABLE NITROGEN	
		p.p.m.		*p.p.m.*		*p.p.m.*	
1	Untreated soil	10.0		31.5		41.5	ave. 41.5
2	Untreated soil	....		....		....	
3	Partially sterilized soil	72.2		6.6		78.8	78.8
4	Partially sterilized soil	70.5		8.3		78.8	
5	Partially sterilized soil, protozoan culture added	58.9 58.9	ave. 58.9	14.1 13.3	ave. 13.7	72.1	71.7
6	Partially sterilized soil, protozoan culture added	58.1		13.3		71.4	
7	Partially sterilized soil, bacterial culture added	68.9		12.4		81.3	81.7
8	Partially sterilized soil, bacterial culture added	68.0 67.2	67.6	14.1 14.9	14.5	82.1	
9	Partially sterilized soil, fungus culture added	58.1		11.6		69.7	68.4
10	Partially sterilized soil, fungus culture added	57.2		10.0		67.2	
11	Partially sterilized soil, soil infusion added	5.8		78.0		83.8	85.4
12	Partially sterilized soil, soil infusion added	7.5		79.6		87.1	

From these results, taken together with the two similar experiments, one can scarcely doubt that the protozoa have had a depressing effect on the CO_2 evolution from the soil as well as on the bacterial numbers. It must be pointed

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[4] The data of the CO_2 evolution experiments were checked by R. A. Fisher, Sc.D., of the Statistical Department of Rothamsted and were found to be clearly significant in regard to the greater evolution of CO_2 by bacteria than by protozoa and bacteria.

TABLE 7

The effect of reinoculation of protozoa and bacteria into partially sterilized soil on the CO_2 evolution from 200 gm. moist soil

Milligrams C as CO_2

DAYS	PROTOZOA						BACTERIA					
	1*	3	5	7	9	11	2	4	6	8	10	12
6	9.7	9.6	9.6	9.6	9.4	9.6	10.1	9.8	9.7	9.9	9.2	9.6
6	5.8	6.8	8.6	8.0	7.3	7.2	9.0	7.3	9.0	8.8	7.0	7.4
6	6.3	6.8	6.6	6.7	6.6	6.5	7.8	7.4	7.0	8.4	7.3	7.8
6	3.2	2.5	3.2	2.5	2.8	2.8	4.0	2.7	3.9	3.6	3.5	3.4
6	2.4	2.2	2.7	2.3	2.0	1.9	3.4	3.2	2.3	2.7	2.3	2.8
6	2.0	2.3	2.3	2.1	2.0	2.0	2.7	2.7	2.1	2.0	2.3	2.2
6	2.9	2.1	2.3	2.3	2.4	2.1	2.7	2.3	2.1	?	2.3	2.0
6	3.2	2.7	2.8	2.9	3.2	3.5	2.7	3.1	2.1	3.5	3.4	3.2
6	2.8	1.8	2.4	3.8	3.6	2.7	3.1	3.2	3.7	2.8	3.9	2.5
Total 54	38.3	36.8	40.5	40.2	39.3	38.3	45.5	41.7	41.9	43.9†	41.3	40.9

* Flask number.

† Average of other 5 flasks taken for missing 6-day value.

TABLE 8

Effect of protozoa when reinoculated into partially sterilized soil, on the accumulation of available nitrogen (nitrate and ammoniacal)

FLASK NUMBER	REINOCULATING AGENT	INCUBATION PERIOD	AVAILABLE NITROGEN		
		days	*p.p.m.*		
1	Protozoa	75	101.6 103.2	ave. 102.4	ave. 103.4
3	Protozoa	75	104.0 104.8	104.4	
2	Bacteria	75	113.6 113.6	113.6	113.0
4	Bacteria	75	112.0 112.8	112.4	
5	Protozoa	90	115.2	116.2	
7	Protozoa	90	124.0		
9	Protozoa	90	108.0		
11	Protozoa	90	105.6		
6	Bacteria	90	124.0	122.8	
8	Bacteria	90	131.2		
10	Bacteria	90	115.2		
12	Bacteria	90	120.8		

out, however, that the effect has been a small one. Likewise the conclusion is inevitable that under the condition of the experiments the amount of available nitrogen accumulating has been reduced by the action of *Hartmanella hyalina*. Here again the amounts are very small but, it is believed, beyond experimental error.

On the other hand, it is shown that the two fungi tested also have had an effect, fully as large as that of the protozoa. The CO_2 evolution has been stimulated and the amount of accumulating nitrogen decreased. This is in accordance with our knowledge of the physiology of the fungi, which decompose considerable organic matter including cellulose, as indicated by the evolution of CO_2; this is always accompanied by a considerable synthesis of cell substance and therefore by a considerable assimilation of nitrogen by the organisms (19). As a result, fungi liberate less available nitrogen (as ammonia) for a definite amount of organic matter decomposed.

It is interesting to note that some unpublished work by Cutler and Crump (6) on sterilized soil confirms these results with regard to CO_2 depression by *Hartmanella hyalina*. These workers added glucose to their soils, which were then inoculated with a single species of bacteria and with the same bacteria associated with the amoebae.

DISCUSSION

It is well established that partial soil sterilization by heat or volatile antiseptics results in the following phenomena:

1. Increase in evolution of CO_2.
2. Increase in accumulation of available nitrogen (as ammonia).
3. Decrease in numbers of bacteria followed by increase to numbers greater than original.
4. Decrease in numbers of protozoa, sometimes in their entire suppression, especially the ciliates and amoebae.
5. Killing of nitrifying bacteria.
6. Killing of worms and other invertebrates, some of which acted as pests.
7. Decrease in numbers of fungi as determined by plate counts, and sometimes in their entire suppression, some of these having acted as parasites.
8. Increase in plant growth of following crop.

Russell and Hutchinson's ingenious protozoan theory whereby the partial sterilization phenomena were explained by the phagocytic action of the soil protozoa, which is prevented by partial sterilization, is to some extent supported by the experiments recorded in this paper. The CO_2 evolution and NH_3 accumulation as well as the bacterial numbers were checked slightly by *Hartmanella hyalina*, a common soil amoeba. One can safely say, it is believed, that protozoa, at least under the conditions obtaining at Rothamsted, constitute *one of the factors* which are to be taken into account in studying partial sterilization. This is in accordance with the opinion previously expressed by Cutler (32).

It must be confessed, however, that the writer cannot admit that the suppression of the protozoa is the sole or even the most important factor responsible for partial sterilization phenomena. Waksman and Starkey (42) and Lebedjanzev (20, 21, 22, 23) have shown that air drying of soils gives a similar rise in bacterial numbers, CO_2 evolution, and accumulation of available nitrogen, and increased plant growth, without destruction of protozoa. Then too, the workers on partial sterilization have nearly all ignored the other members of the soil population, the fungi, algae, actinomycetes, and the invertebrate fauna. The results obtained by the writer show an action on the part of fungi which is as large as or larger than that of the protozoa studied. The theory of Störmer (37) supported by the data of Waksman and Starkey (42) and others must be considered. According to this theory the increased development of bacteria, evolution of CO_2, and accumulation of available nitrogen in partially sterilized soils are due to the killing of most of the fauna and flora, which are subsequently attacked and decomposed, thus releasing the protoplasmic nitrogen and carbon as NH_3 and CO_2. The bacteria which decompose these organisms use only a small portion of this organic matter for resynthesis into bacterial cells. The data presented above are in accordance with this theory which, however, should perhaps be qualified to make place for the action of the protozoa on soil processes. Thus the protozoan theory of Russell and Hutchinson may not be in opposition to the earlier theory of Störmer but a modification of it.

It must also be noted that only one of the two or three common soil amoebae, and of the five or six species of protozoa numerous in soils, was considered. The depressive effect might well be intensified by the action of these other species although one would not expect much from the small flagellates, especially as some are not known to feed on bacteria. The depressive action of *Hartmanella hyalina* on so-called "ammonification" and "soil respiration," can no doubt be accounted for by the locking up in their bodies, as microbial protoplasm, of some of the carbon and nitrogen that otherwise would have become available as CO_2 and NH_3. This seems more probable when we consider that the difference between the evolution of CO_2 from flasks of soils reinoculated with and without protozoa is only noticed in the first weeks of the experiments. Also it should be noted that the soil is a heavy clay and rather moist. Koffman (17) and Cutler and Dixon (8), however, found active protozoa in most types and conditions of soil, even when fairly dry.

In regard to the results from the fungi it is of great interest to note that the depression of NH_3 accumulation was greater in flasks inoculated with fungi than in flasks inoculated with protozoa, and the stimulation of CO_2 evolution by fungi was greater than the depression caused by protozoa. In both cases, in other words, the action of the fungi was greater than that of the protozoa. It would seem that Waksman and Starkey (42) are correct in attributing as great or greater importance to the fungi than to the protozoa in partial sterilization phenomena. Inasmuch as only two species of the large soil fungus population was studied, one cannot draw any definite conclusions. As in the case of the protozoa, one would expect a larger action from the whole population than from the two species studied, although this does not necessarily follow.

For full discussion of the extensive literature on partial sterilization and soil protozoa, the reader is referred to the critical works of Cutler (32), Kopeloff, Lint, and Coleman (18), Russell (31), and Waksman and Starkey (42).

Since these reviews have appeared, still another theory has been proposed, which might be mentioned at this point. Matthews (27), would explain the increase in numbers of bacteria in soils partially sterilized by antiseptics as being due to the increase in available food from the antiseptics themselves. Unfortunately this theory is contrary to the known principles of microbiological physiology. It is well established that when available non-nitrogenous materials are added to a soil or artificial medium, the extensive microbial population that develops must obtain its necessary nitrogen from other compounds of the soil or culture medium. This may lead, therefore, to a decrease in available nitrogen. In no case does nitrate or ammoniacal nitrogen accumulate to the extent that it does in partially sterilized soil. If, on the other hand, the non-nitrogenous "food" acts as a source of energy for nitrogen fixation, it does so only after the supply of available nitrogen in the soil has been used up; ammonia or nitrates do not accumulate, but, on the contrary, decrease. After all the added non-nitrogenous material has been decomposed, nitrogen may again accumulate, but for long periods of time the amount of this nitrogen is less than in soil to which no such compounds have been added. Partial sterilization results are so contrary to these facts that Matthews' theory cannot be accepted at present. Her observations that the antiseptics may be used by bacteria as a source of energy are of great interest. However, any increase in numbers of bacteria due to utilization of non-nitrogenous antiseptics must be accompanied by a transformation of available nitrogen in the soil into microbial cell substance. Even agreeing with Matthews that "the increased fertility observed by Russell and Hutchinson is to be attributed in large measure to the activity of the greater population in breaking down the organic matter of the soil," but considering the work of Störmer (37) and Waksman and Starkey (42), one may suggest that, whereas the antiseptics might have caused an increase in numbers of bacteria, the increased fertility and the greater part of the increase in numbers of bacteria are due, not to a stimulation of bacteria into greater activity, but to the killing of the large soil population of living cells, thus making them available for decomposition. Any increase in numbers of bacteria due to utilization of the non-nitrogenous antiseptics would tend to neutralize the increased fertility due to decomposition of the killed soil population, for the nitrogen from the latter would be reabsorbed into microbial cells necessary to decompose the antiseptic. The fact that treatment by heating soils or by air drying them gives results similar to those obtained by treatment with antiseptics also speaks against her theory, as an explanation for partial sterilization phenomena. Also it must be remembered that the physical effects of such drastic treatment and the biological effect of destroying various parasites are in many cases of prime importance (18, 42).

SUMMARY

1. Soil was partially sterilized by heating and reinoculated with various agents.

2. *Hartmanella hyalina* caused a reduction in the number of bacteria and a slight depression in CO_2 evolution and NH_3 accumulation.

3. *Trichoderma köningi* and *Penicillium* sp. caused an increase in CO_2 evolution and a decrease in NH_2 accumulation.

4. Partial sterilization phenomena are discussed in relation to these findings.

REFERENCES

(1) Allison, R. V. 1924 A note on the protozoan fauna of the soils of the United States *Soil Sci.* 18: 339–352.

(2) Cunningham, A. 1914 Studies on soil protozoa: II. *Centbl. Bakt.* (II) 42: 8–27. Also *Jour. Agr. Sci.* 7: 49–74.

(3) Cutler, D. W. 1920 A method for estimating the number of active protozoa in the soil. *Jour. Agr. Sci.* 10: 135–143.

(4) Cutler, D. W. 1923 The action of protozoa on bacteria when inoculated into sterile soil. *Ann. Appl. Biol.* 10: 137–141.

(5) Cutler, D. W., and Crump, L. M. 1920 Daily periodicity in numbers of active soil flagellates: with a brief note on the relation of trophic amoebae and bacterial numbers. *Ann. Appl. Biol.* 7: 11–24.

*(6) Cutler, D. W., and Crump, L. M. Unpublished data. (To be published in *Ann. Appl. Biol.*)

(7) Cutler, D. W., Crump, L. M., and Sandon, H. 1922 A quantitative investigation of the bacterial and protozoan population of the soil with an account of the protozoan fauna. *Phil. Trans. Roy. Soc. (London) Ser. B.* 211: 317–350.

*(8) Cutler, D. W., and Dixon, A. Unpublished data. (To be published in *Ann. Appl. Biol.*)

(9) Fellers, C. R., and Allison, F. E. 1920 The protozoan fauna of the soils of New Jersey. *Soil Sci.* 9: 1–25.

(10) Goodey, T. 1916 Further observations on protozoa in relation to soil bacteria. *Proc. Roy. Soc. (London) Ser. B.* 89: 297–314.

(11) Greig-Smith, R. 1912 Contribution to our knowledge of soil fertility: VI. The inactivity of the soil protozoa. *Proc. Linn. Soc. New South Wales* 37: 655–672.

(12) Harper, H. J. 1924 The determination of ammonia in soils. *Soil Sci.* 18: 409–418.

(13) Hills, T. L. 1916 The relation of protozoa to certain groups of soil bacteria. *Jour. Bact.* 1: 423–452.

(14) Hiltner, L., and Störmer, K. 1903 Studien über die Bakterienflora des Ackerbodens, mit besonderer Berucksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. *Arb. K. Gsndhtsamt. Biol. Abt.* 3: 445–545.

(15) Koch, G. P. 1915 Activity of soil protozoa. *Jour. Agr. Res.* 5: 477–488.

(16) Koch, G. P. 1916 Studies on the activity of soil protozoa. *Soil Sci.* 2: 163–181.

*(17) Koffman, M. Beiträge zur Kenntnis der Bodenprotozoen. (To be published in *Acta Zoologica* Stockholm.)

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* Personal conversation with author; manuscripts not read.

(18) Kopeloff, N., Lint, H. C., and Coleman, D. A. 1916 A review of investigations in soil protozoa and soil sterilization. *Centbl. Bakt.* (II) 46: 28–74. Also *Soil Sci.* 3: 197–269, 1917.

(19) Kruse, W. 1910 Allgemeine Mikrobiologie. Leipzig.

(20) Lebediantzeff, A. 1924 Sur la dessiccation des sols agricoles à l'air libre. *Compt. Rend. Acad. Sci.* 178: 793–795.

(21) Lebediantzeff, A. 1924 Modifications subies par la terre vegetale sechée à l'air. *Compt. Rend. Acad. Sci.* 178: 960–963.

(22) Lebediantzeff, A. 1924 Sur la reparition de la fertilité dans la couche arable selon la profondeur. *Compt. Rend. Acad. Sci.* 178: 1381–1383.

(23) Lebedjanzev, A. N. 1924 Drying of soil as one of the natural factors in maintaining soil fertility. *Soil Sci.* 18: 429–447.

(24) Lipman, J. G., Blair, A. W., Owen, I. L., and McLean, H. C. 1910. Experiments relating to the possible influence of protozoa on ammonification in the soil. N. J. Agr. Exp. Sta. Bul. 248.

(25) Martin, C. H., and Lewin, K. R. 1914 Some notes on soil protozoa. *Phil. Trans. Roy. Soc. (London) Ser. B.* 205: 77–94.

(26) Martin, C. H., and Lewin, K. R. 1915 Notes on some methods for examination of soil protozoa. *Jour. Agr. Sci.* 7: 106–119.

(27) Matthews, A. 1924 Partial sterilization of soil by antiseptics. *Jour. Agr. Sci.* 14: 1–57.

(28) Moore, G. T. 1912 Microörganisms of the soil. *Science.* n.s. 36: 609–616.

(29) Nasir, S. M. 1923 Some preliminary investigations on the relationship of protozoa to soil fertility with special reference to nitrogen fixation. *Ann. Appl. Biol.* 10: 122–133.

(30) Perey, M. 1922 Les protozoaires du sol. Etat actuel de la question et quelques recentes observations. *Ann. Sci. Agron.* 39: 331–352.

(31) Russell, E. J. 1921 Soil Conditions and Plant Growth, ed. 4. London.

(32) Russell, E. J., et al. 1923 The Microörganisms of the Soil. London.

(33) Russell, E. J., and Hutchinson, H. B. 1909 The effect of partial sterilization of soil on the production of plant-food. *Jour. Agr. Sci.* 3: 111–144.

(34) Russell, E. J., and Hutchinson, H. B. 1913 The effect of partial sterilization of soil on the production of plant-food: II. The limitation of bacteria numbers in normal soils and its consequences. *Jour. Agr. Sci.* 5: 152–221.

*(35) Sandon, H. Unpublished data.

(36) Sherman, J. M. 1916 Studies on soil protozoa and their relation to the bacterial flora. *Jour. Bact.* 1: 33–66, 165–185.

(37) Störmer, K. 1907 Über die Wirkung des Schwefelkohlenstoffs und ähnlicher Stoffe auf den Boden. *Jahresber. Angew. Bot.* 1908: 113–131.

(38) Thornton, H. G. 1922 On the development of a standardized agar medium for counting soil bacteria with especial regard to the repression of spreading colonies. *Ann. Appl. Biol.* 9: 241–274.

(39) Waksman, S. A. 1916 Studies on soil protozoa. *Soil Sci.* 1: 135–152.

(40) Waksman, S. A. 1916 Protozoa as affecting bacterial activity in soils. *Soil Sci.* 2: 363–376.

(41) Waksman, S. A., and Fred, E. B. 1922 A tentative outline of the plate method for determining the number of microörganisms in the soil. *Soil Sci.* 17: 27–28.

(42) Waksman, S. A., and Starkey, R. L. 1923 Partial sterilization of soil, microbiological activities, and soil fertility. *Soil Sci.* 16: 137–157, 247–268, 343–357.

(43) Waksman, S. A., and Starkey, R. L. 1924 Microbiological analysis of soil as an index of soil fertility: VII. Evolution of carbon dioxide. *Soil Sci.* 17: 141–161.

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*Personal conversation with author; manuscripts not read.

THE RELATIVE ALUMINUM TOLERANCE OF CROP PLANTS[1]

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INTRODUCTION

The toxicity of aluminum to plants and its activity in acid soils have been clearly demonstrated (1, 3, 6).

The comparative resistance of plants to aluminum poisoning has been predicted from field observations (2) but few tests have been made with definite magnitudes of aluminum in solutions. Solutions of inorganic compounds of aluminum, such as nitrate, sulfate, and chloride readily form precipitates of $Al_2(PO_4)_2$, if phosphate is present in the solution, or of hydroxide, if the solution is not strongly acid. In the absence of phosphates, considerable aluminum can be maintained in solutions at a hydrogen-ion concentration of between pH 4.0 and 4.5. Taking advantage of this fact, Stoklasa (9) grew wheat plants in cultures supplied alternately with phosphate and with aluminum and manganese salts. In the present study, a similar technique was used in testing the relative tolerance of different crop plants for different magnitudes of aluminum in solution. The plants were grown during half of each week in a complete nutrient solution; then during the remaining half of the week, they were put into solutions lacking only the phosphate salts. To this latter phosphorus-free solution was added the desired amounts of aluminum in an $Al_2(SO_4)_3$ solution. In this way it was found possible to grow a variety of plants in culture solutions ranging in hydrogen-ion concentration from pH 4.0 to 5.0, and containing considerable amounts of aluminum in solution. Further, the plants were supplied with phosphorus separately from the aluminum and there could be little question of the aluminum rendering the phosphorus unavailable in the solution outside the plants.

The results of this study appear to show that aluminum in appreciable amounts in solution is toxic to most plants, causing at first a dwarfing and distortion of the roots, and later injury to the entire plant. The injury to the roots is most marked in crops that are most susceptible to aluminum toxicity. A study of one crop (corn) further showed that the greatest accumulation of aluminum is in the roots. The aluminum was found further to be localized in the cortex of the roots immersed in the solution. In the living cells it is accumulated in the protoplasm, rather than in the cell walls or in the vacuoles, and is especially abundant in the nucleus, as shown by haematoxylin tests (4). A cursory examination of a few roots of cabbage, peppers, redtop, lettuce, and timothy showed similar localization of aluminum.

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[1] Contribution no. 339 of the station.

EXPERIMENTAL DATA

The first cultures grown with this procedure were of rye of the Rosen variety. The method of handling the plants was the same as that subsequently employed in all of these experiments and was as follows: All plants were grown on the bench of a well-lighted greenhouse. Seed was sown on a net suspended over a graniteware dish of tap water and allowed to remain until the plants were 5 cm. high. They were then mounted on perforated paraffined corks, 6 plants to each cork, and placed in 250-cc. bottles of nutrient solution. Precautions were taken to darken the bottles to prevent algal growth in the solution.

The rye plants were set in 250-cc. jars on March 6, 1924, and supplied with a basal solution of the following composition: $MgH_4(PO_4)_2$—0.00016 *M*, KNO_3—0.002 *M*, NH_4NO_3—0.002 *M*, $CaSO_4$—0.0001 *M*, and $Fe(NO_3)_3$—0.00004 *M*. This was designated as solution L, and was supplied to fifteen of the cultures on Monday of each week. On the following Thursdays, 5 cultures received a similar solution, but with $MgSO_4$ replacing $MgH_4(PO_4)_2$ in equivalent concentrations; 5 others received the same solution with the addition of 3.5 p.p.m of aluminum as $Al_2(SO_4)_3$; and another lot of 5 received 12.8 p.p.m. of aluminum as $Al_2(SO_4)_3$. The greater acidity of the aluminum-containing solutions was counteracted by additions of Na_2CO_3 solution, so that the acidity of all cultures at the beginning of each period was approximately pH 4.5. This treatment was continued until May 15, when the plants were removed and dried.

A second series was grown in a slightly different nutrient solution, composed of $MgH_4(PO_4)_2$—0.00016 *M*, $(NH_4)_2$ SO_4—0.0025 *M*, KCl—0.0008 *M*, $CaSO_4$ —0.0008 *M*, and $Fe(NO_3)_3$—0.00004 *M*. This solution was designated solution M. This latter solution regularly became more acid, because of the removal of ammonia from the $(NH_4)_2 SO_4$ and the plants made a decidely poorer growth than in solution L. The average yields are given in table 1.

The growth of the plants in both cultures as shown in the table 1 was depressed by 3.5 p.p.m. of added aluminum and severely injured by 12.8 p.p.m. The roots showed greater injury than the tops, those of the solution L cultures being reduced to 41 per cent of the control plants, whereas the tops were only reduced to 57 per cent by 12.8 p.p.m. of aluminum. The aluminum content of the plants was slightly increased by the aluminum treatment. Rye is regarded as relatively resistant to aluminum toxicity (2).

Solution cultures were grown in the greenhouse during the winters of 1924–25 and 1925–26. Plants were germinated on a perforated aluminum plate floated on corks over tap water. When about 5-cm. high, the seedlings were transferred to perforated paraffined corks and mounted over 250-cc. jars. In the case of large plants, such as cabbage and corn, one plant was put in each jar; with smaller seedlings, 6 plants were grown in each 250-cc. jar.

The basal nutrient solution used in this and the subsequent cultures was as follows: $MgSO_4$—0.0008 *M*, $Ca(NO_3)_2$—0.0015 *M*, NH_4NO_3—0.001 *M*, KCl—0.0008 *M*, $Fe(NO_3)_3$—0.000008 *M*.

This solution was changed twice weekly. During half of each week, 0.000066 *M* monocalcium phosphate was supplied. During the remaining half of each week, the desired amount of aluminum was supplied, and the acidity of the different culture solutions was adjusted by the addition of dilute sulfuric acid or of sodium carbonate, so that all of the solutions of one series were of similar acidity and between pH 4.0 and 4.5.

Table 2 shows that lettuce proved very sensitive to aluminum toxicity. One and a half parts per million of aluminum added to the solution produced a notable depression in yield.

All the cultures were grown with tap water from a surface well. With this it was easy to maintain a suitable acidity because of the buffering action of the salt content of the tap water.

TABLE 1

Yields of Rosen rye from solution cultures 70 days old*

	FROM SOLUTION L		FROM SOLUTION M			
	Weight of tops	Weight of roots	Weight of whole plant	Proportion in dry matter of		
				P_2O_5	Al_2O_3	Fe_2O_3
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*
Solution alone	18.02	4.76	13.00	1.04	0.05	0.10
Solution plus 3.5 p.p.m. aluminum	14.76	3.59	10.91	1.40	0.07	0.15
Solution plus 12.8 p.p.m. aluminum	10.35	1.94	8.13	1.73	0.12	0.09

* 30 plants.

Not all of the cultures were grown at the same time and there were wide variations in results from successive tests with the same kinds of plants. Despite this, the crops can be tentatively grouped into three classes:

Sensitive (depressed by 2 p.p.m. of added aluminum)—lettuce, beets, timothy, and barley.

Medium sensitive (depressed by 7 p.p.m. of added aluminum or less)—radishes, sorghum, cabbage, oats, and rye.

Resistant (depressed by 14 p.p.m. of added aluminum or more)—corn, turnips, and redtop.

The relative tolerance to aluminum is in noticeably close agreement with the field observations of Burgess and Pember (2) of resistance to acid-soil conditions, and with the results of Hartwell and Pember (3) for barley and rye. The only conspicuous discrepancy is in the case of carrots, which appear to be quite tolerant of acid-soil conditions in the field, but which in solution cultures were seriously injured by low concentrations of aluminum. This observation is based, however, on only 1 series of cultures including carrots, and must be substantiated by further tests before it can be regarded as at all certain.

ACIDITY OF SOLUTION

It has been found repeatedly by numerous workers that growing plants have a tendency to change the acidity of the culture solutions in which they are grown. Since aluminum is easily precipitated from solution by slight reductions of acidity below pH 4.5, it was thought that possibly the apparently tolerant plants may have made the culture solutions more alkaline during the 3.5-day period of exposure to them and thus have rendered the aluminum non-injurious by precipitating it. Several preliminary tests were made during the growth of the earlier cultures in 1924, indicating that lettuce tended to make the culture solution more acid and that radishes and rye tended to make it more alkaline. On April 17, more comprehensive tests were made on the 1925 cultures when the majority of the culture plants had been grown in solution for about 6 weeks, and had been for 2 days in the particular solution tested.

TABLE 2

Yields of lettuce in solution cultures grown with different concentrations of aluminum, expressed as relative values with controls as 100

KIND OF PLANT	NUMBER OF PLANTS	AGE OF CULTURE	AIR-DRY WEIGHT PER PLANT WITHOUT ALUMINUM								
		weeks		*gm.*							
Concentration of aluminum, *p.p.m.*					0.54	0.9	1.08	1.62	1.8	2.7	3.6
Lettuce, May King	36	8	Whole plant	0.28	..	96	..	..	61	51	45
Lettuce, May King	30	6	Roots	0.072	98	..	89	76	..	..	..
			Tops	0.312	80	..	75	54	..	..	..
Lettuce, Cos	36	8	Whole plant	0.028	..	99	..	..	82	45	25
Concentration of aluminum. *p.p.m.*					0.7	1.4	2.2	2.9	..	..	..
Lettuce, May King	30	9	Roots	0.33	99	85	79	65	..	..	..
			Tops	0.82	98	92	82	70	..	..	..

The following were the average pH values of the solutions from four or more cultures each of different crops, as determined by the use of Clarke and Lubbs indicator dyes:

Sensitive crops—Lettuce 3.8, beets 6.3, timothy 3.6, and barley 6.4.
Medium sensitive crops—Radishes 6.0, sorghum 3.9, cabbage 6.5, oats 6.5, and rye 6.8.
Resistant crops—Corn 6.7, turnips 6.1, and redtop 3.6.

The crops which had a neutralizing influence on the culture solution included the sensitive, beets and barley; medium, cabbage, radishes, turnips and rye; and resistant, corn and oats. The crops having an acidifying influence were likewise well distributed in the different groups, one of the most sensitive crops, lettuce, and the most resistant, redtop, both being strong acidifiers. The tendency of the crops to acidify or neutralize the solution, therefore bore no apparent relation to the tolerance of that crop to aluminum poisoning.

The toxicity of the $Al_2(SO_4)_3$ is irrespective of the solution becoming more or less acid because of the growth of the plants. The previous cultures of rye, in solution M, which were rendered acid by the rye alone, also showed the toxic action of aluminum, even though the aluminum-containing cultures were not more acid than the controls without aluminum. The aluminum itself was apparently strongly toxic in any culture solution in which it could remain dissolved. This toxicity could not be due to precipitation of phosphate from the culture solution, since phosphate and aluminum salts were supplied alternately. This is in opposition to the conclusion of Line (5), that depression by aluminum in culture solutions is due either to precipitation of phosphate or to acidity. In these cultures the depression in plant growth would appear to be due to the toxicity of the aluminum itself.

MANIFESTATIONS AND LOCALIZATION OF ALUMINUM POISONING

All of the crops that were at all depressed by aluminum showed the first evidences of injury in the roots. These at first became discolored, then branch roots became stunted, or failed to develop, and the whole root system became dwarfed. These symptoms were apparent long before there was any visible depression in the growth of the tops. In the case of very sensitive crops, such as beets and lettuce, the roots died promptly in solutions containing appreciable quantities of aluminum. The roots of corn and other noticeably resistant crops were deformed, but persisted, except in the higher concentrations of aluminum (plate 1, fig. 1). Redtop was apparently uninjured by rather strong solutions of aluminum, and even a concentration of more than 50 p.p.m., which depressed the yield a little, did not visibly injure or deform the roots. Thus the aluminum poisoning appeared to be localized in the roots.

Similar observations of root injury due to aluminum have been noted by McGeorge (6), Magistad (7), Hoffer (4), Rothert (8), and others. Since, primarily, aluminum injury is in the roots, the effect of aluminum treatment on the absorption of dyes, nutrients, and water by corn roots was tested. The first tests were made on January 6, 1926, with corn which were about 1 foot tall and had been grown in solution since November 16, 1925. Six cultures of 2 plants each were tested, 3 from cultures receiving 21.6 p.p.m. of aluminum

TABLE 3

Yields of various plants in solution cultures grown with different concentrations of aluminum, expressed as relative values with controls as 100

KIND OF PLANT	NUMBER OF PLANTS	AGE OF CULTURE	AIR-DRY WEIGHT PER PLANT WITHOUT ALUMINUM									
		weeks		gm.								
Concentration of aluminum, *p.p.m.*					1.8	3.6	7.2	14.4	21.6	28.8	43.2	57.6
Beets, Early Wonder	30	18	Whole plant	1.28	26	12	16	..	..	..	..	..
Beets, Early Wonder	10	11	Whole plant	1.00	74	23	35	..	..	..	..	..
Beets, Mangel	30	18	Whole plant	2.34	75	38	25	..	..	..	..	..
Carrots, Chantenay	5	18	Whole plant	4.03	..	26	13	10	..	8	..	..
Carrots, Chantenay	5	18	Whole plant	3.45	..	23	29	13	..	9	..	..
Radish, Scarlet Globe	6	11	Roots	0.53	106	79	49	62	..	..	..	..
			Tops	0.54	106	79	52	62	..	..	..	..
Turnip, White Globe	5	11	Roots	0.3	283	487	273	107	..	..	..	..
			Tops	0.85	78	81	61	44	..	..	..	..
Cabbage	30	18	Whole plant	0.69	..	93	94	112	..	..	..	..
Cabbage	10	14	Whole plant	2.04	..	..	57	48	..	34	38	..
Barley	30	11	Roots	0.47	53	43	51	44	..	..	..	..
			Tops	0.86	78	69	70	65	..	..	..	..
Oats	30	9	Roots	0.11	..	96	74	73	..	..	..	..
			Tops	0.47	..	92	78	74	..	..	..	..
Oats	30	10	Roots	0.19	99	121	141	147	..	..	..	..
			Tops	0.68	86	78	124	129	..	..	..	..
Rye	30	9	Roots	0.17	78	63	62	..	..	..	..	..
			Tops	0.31	88	87	75	..	..	..	..	..
Rye	30	10	Roots	0.36	79	85	77	99	..	..	..	..
			Tops	0.68	80	70	91	119	..	..	..	..

TABLE 3—*Continued*

KIND OF PLANT	NUMBER OF PLANTS	AGE OF CULTURE	AIR-DRY WEIGHT PER PLANT WITHOUT ALUMINUM									
		weeks		*gm.*								
Corn, R. I. White Cap....	5	5	Roots	2.0	..	101	101	75	..	..	..	..
			Tops	6.0	..	77	92	67	..	..	..	..
Corn, R. I. White Cap....	4	10	Roots	1.54	..	45	82	45	36	..	..	..
			Tops	9.48	..	102	92	63	61	..	..	..
Sorghum................	30	10	Roots	0.36	44	56	49	49	..	..	..	..
			Tops	0.54	64	64	90	69	..	..	..	..
Timothy................	24	10	Roots	0.17	78	73	40	..	..	..	..	..
			Tops	0.63	65	70	51	..	..	..	..	..
Redtop..................	24	10	Whole plant	0.50	129	112	136	..	..	72	..	84

in $Al_2(SO_4)_3$ and no P_2O_5 one-half of each week, the other 3 having had nutrient solutions without aluminum, but with alternating phosphorus and no phosphorus. At 10 a.m. the roots of one aluminum treated and one non-aluminum treated culture were immersed in solutions of malachite green, rosolic acid, and water-soluble eosin, respectively. At 2 p.m. these were removed from the dye and examined.

Of the control plants, eosin stained all of the leaves to the very tips. Of the aluminum-treated plants, one had eosin stain in the veins of the leaf sheath of the largest leaf, 1.5 inches above the roots, the second had stain in the vessels of the four outer-leaf sheaths 4 inches above the roots, but no further out into the leaves. Malchite green stained the cortex but the stain did not penetrate beyond that point in either the aluminum-treated or the control plants. Rosolic acid stained the outside of the roots of the aluminum plants, but not those of the controls, and did not penetrate into the tissues of either. Thus the aluminum treatment caused the epidermis of the roots to stain more readily with rosolic acid, and hindered penetration of eosin into the tissues.

Similar tests with material from the same set of cultures were made on January 8, after the cultures had been for 1 day on uniform culture solutions without aluminum. The roots were immersed in 0.001 per cent methyl violet and in 0.1 per cent eosin for 1 hour, then examined. Eosin had travelled 1.5 inches up the petioles in the control plants and had not penetrated beyond the cortex of the aluminum-treated plants. Methyl violet did not penetrate beyond the outer cells of the cortex. Other plants were left in the eosin 4 hours, during which time the eosin had penetrated the control plants a distance of over 1 foot to the very tips of the leaves, but had ascended in the aluminum-treated plants only a distance of 7.5 inches above the root collars.

The toxic action of the aluminum was apparently of such a character as to diminish the rate of movement of eosin through the conducting tissues of the plant either by interfering with absorption or by precipitating the dye in the outside tissues. Rosolic acid stained the aluminum-treated roots more strongly than those of the controls.

To learn whether the absorption of nutrients was affected in the same way as that of the dyes, corn plants were deprived of nitrates for several days, then placed in a solution of 0.012 *M* $Ca(NO_3)_2$ for 72 hours. A test was made January 18 from 10.30 a.m. to 5 p.m., when nitrates were found 2 inches up the stem of the control plants, but none in the aluminum-treated plants. On January 21, at 3 p.m., others of this same lot of plants were sectioned, and tested for nitrate with the diphenylamine test. The control plants showed much nitrate in root collar and leaf sheaths, 10 cm. above the roots. The aluminum-treated plants tested at the same time gave a nitrate test only on the surface of the immersed roots.

Lettuce plants grown in solution cultures with different amounts of aluminum were immersed in solutions of eosin and rosolic acid, respectively, from 9 a.m. on January 24 to 9 a.m. on January 25. Then the eosin had penetrated the control plants throughout their tissues, and had reached only to the root collar in the plants subjected to 0.54, 0.90, 1.26, and 2.16 p.p.m. of added aluminum, but the culture treated with 1.62 p.p.m. of aluminum showed as great a penetration of eosin as did the control. The rosolic acid gave the same results as with corn, staining the aluminum-treated roots, but not those of the control plants. Only the plants subjected to the 2.16 p.p.m. of aluminum were conspicuously dwarfed by the aluminum poisoning. Similar tests were made with lettuce in eosin solution on January 27 to 28, and the aluminum-treated and control plants absorbed the dye readily, showing no evident differences. The distribution of the eosin was not so easily followed in the lettuce as in the corn, because of the milky latex.

Two lots of 4 plants each of corn which had been grown 1.5 months in solutions without aluminum were then subjected for 12 days to solutions without phosphorus, but in one case without aluminum and in the other with 21.6 p.p.m. of aluminum in solution. Their rates of water absorption were then measured by the amounts of liquid necessary to refill the jars, for a period of 23 days, with the following results:

	TOTAL LEAF AREA	TOTAL WATER LOSS	WATER LOSS PER SQUARE METER PER DAY
	sq. m.	*cc.*	*cc.*
No aluminum	1.11	713	27.9
21.6 p.p.m. aluminum	1.16	443	16.6

The water absorption by the plants subjected to aluminum was reduced 40 per cent as compared to the control plants of about the same size.

Thus the effect on Rhode Island White Cap corn of aluminum at a concentration which did not greatly dwarf the plants, was such as to make the root surfaces stain more readily with rosolic acid; to decrease very markedly the rate of penetration of eosin and of nitrate ions through the tissues; and to depress the rate of water absorption to 40 per cent from that of normal plants not subject to aluminum.

Such marked effects on absorption would indicate that the aluminum had seriously decreased the permeability of the roots. Stoklasa (10), McGeorge (6), Rothert (8), and others have shown that there is an aluminum accumulation in the roots of most plants. Accordingly tests were made with the haematoxylin method applied by Hoffer and Carr (4) to demonstrate the accumulation of aluminum. Roots of corn, cabbage, and redtop grown with and without aluminum were examined in this way.

The haematoxylin stained only the epidermis and a few cells of the outer layer of cortex of the control plants of all three sorts grown without the addition of $Al_2(SO_4)_3$ to the solution. The roots of aluminum-treated plants of both corn and of cabbage were strongly stained with the haematoxylin. Sections of these roots showed the stain to be confined to the cortical cells (plate 1, fig. 2). The protoplasm and especially the nuclei were strongly stained. The roots of the redtop grown in aluminum solutions were not visibly injured, as were those of corn and cabbage, and when tested with haematoxylin did not show the stain in the cortical cells. Only the epidermal cells and a few of the first layer of cortical cells of the redtop roots were stained in either the aluminum-treated or the control plants.

Thus the haematoxylin test indicated an accumulation of aluminum in the cortex of corn and cabbage roots immersed in aluminum solution. There was very little aluminum in the roots of the more resistant redtop. It seems probable that the accumulation of aluminum in the roots is responsible for their decreased absorption of dyes, of nutrients, and of water. The most marked accumulation was in the protoplasm and nuclei, which were stained very deeply. In just what manner the aluminum reacted in the cell protoplasm to bring about the differences in permeability is not certain.

All the foregoing tests were made with cultures in which the aluminum was supplied in $Al_2(SO_4)_3$, and in which the culture solutions were made up of inorganic salts only. Under such conditions, the aluminum was precipitated very readily by changes in acidity toward neutrality or by the presence of phosphates in the solution. Organic compounds of aluminum are less easily precipitated than $Al_2(SO_4)_3$ and it is possible that in ordinary clayey soils aluminum may be combined with organic acids. Accordingly, aluminum citrate and aluminum tartrate were prepared by dissolving precipitated aluminum hydroxide with citric and tartric acid, respectively.

Cultures of Grimm alfalfa were grown in 250-cc. jars from March 16 to June 16, 1926 in the same basal nutrient solution as was used for the previous cultures to test aluminum toxicity. Four plants germinated in sand and transferred to the culture bottles when in their second leaf, were started in each culture. Aluminum treatment was begun on April 1 and solutions were changed twice weekly. Tests were made of the acidity of the culture solutions on April 12, after they had been in the culture vessels 2 days.

Where phosphate was supplied continuously, as in series 3, half the amount was put in each time, so that the same total amount was supplied, whether this all was furnished during half of each week, or continuously. The cultures of series 1 had an initial acidity of about 4.5, of series 2 of about 5.9, and the other cultures were neutralized with Na_2CO_3 to about pH 6.8 to 7.0 at the beginning of each culture period. The aluminum citrate in solution without $CaH_4(PO_4)_2$ remained clear. The aluminum citrate and tartrate solutions with phosphorus became cloudy when neutralized, but no precipitate settled out. All of the cultures containing citrate or tartrate developed a fungus or yeast growth, and became cloudy and foamy a day after they were set up with the plants.

TABLE 4

Test of acidity of culture solutions after alfalfa had grown in them for 2 days

Concentrations of aluminum, *p.p.m.*	0	1.8	3.6	7.2	14.4
1. Alternating $CaH_4(PO_4)_2$ and $Al_2(SO_4)_3$	4.4	4.2	7.0	6.2	6.4
2. Same as above except $Al_2(SO_4)_3$ neutralized partially at beginning	4.6	4.0	6.5	6.4	6.0
3. Continuous aluminum citrate and $CaH_4(PO_4)_2$	7.6−	7.6−	7.6	7.6−	7.6−
4. Alternating aluminum citrate and $CaH_4(PO_4)_2$	7.6−	7.6−	Plants dying		
5. Alternating aluminum tartrate and $CaH_4(PO_4)_2$	7.6−	Plants dying			7.6−

TABLE 5

Weights per plant of alfalfa from solution cultures June 16, 1926

METHOD OF TREATMENT	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Concentrations of aluminum in *p.p.m.*	0		1.8		3.6		7.2		14.4	
1 and 2. Alternating phosphate and $Al_2(SO_4)_3$, half each week	0.44	0.64	0.28	0.50	0.24	0.31	0.11	0.25	0.09	0.21
3. Continuous phosphate and aluminum citrate	0.45	0.80	0.12	0.25	0.29	0.21	Died		0.14	0.08
4. Alternating phosphate and aluminum citrate, half each week	Died just before harvest		Died early		Died early		Died early		Died early	
5. Alternating phosphate and aluminum tartrate	0.32	0.56	Died early		Died early		Died early		0.09	0.12

The alfalfa plants were harvested on June 16, after 2.5 months of aluminum treatment. The lower leaves of all plants had then begun to dry up and fall off.

The weights given in table 5 were based on only 1 to 4 plants in each case and the cultures were not duplicated. The quantitative comparisons are no more than indicative, but certain facts stand out clearly.

Young alfalfa plants rendered the culture solution alkaline so rapidly that aluminum was quite promptly precipitated from sulfate and little toxic effect was produced. Aluminum citrate and aluminum tartrate did not yield precipitates even when the solutions were neutralized, and these were quite strongly toxic to alfalfa. When the phosphate and the aluminum were alternated, even 1.8 p.p.m. of aluminum as citrate or tartrate killed the plants in about 2-weeks' time. The presence of phosphate in the solution at the same time seemed to protect the plants; thus in series 3, in which aluminum citrate and mono-calcium phosphate were both supplied at each change of solution, the plants were not killed and showed only slight depression. The plants from the $Al_2(SO_4)_3$ culture likewise showed little depression, because aluminum was precipitated from the solution, which was rendered alkaline by the presence of alfalfa.

In all of these experiments, the protective influence of phosphates against aluminum poisoning is very evident. In the case of $Al_2(SO_4)_3$, the phosphates precipitated out the aluminum very promptly when the two were put into solutions together. Even aluminum citrate seems to be rendered much less toxic by the presence of phosphates though aluminum was not precipitated.

CONCLUSIONS

1. All crops studied were injured by sufficiently high concentrations of aluminum, if this was supplied alternately with phosphorus, and in a culture solution of an acidity which would not precipitate the aluminum.

2. There were wide variations in the sensitiveness to aluminum of the different crops studied.

(a) The most sensitive crops were lettuce, beets, timothy, and barley.

(b) The medium sensitive crops were radishes, sorghum, cabbage, oats, and rye.

(c) Corn, turnips, and redtop were noticeably resistant to aluminum poisoning, redtop being the most resistant of the three.

3. The tendency of the crops to change the solutions toward a more alkaline or more acid reaction was not correlated with the sensitiveness of the crop toward aluminum.

4. The first evidence of toxicity of aluminum was in the dwarfing and injury to the roots.

5. Aluminum poisoning to corn plants was accompanied by reduced absorption of dyes, of nitrate, and of water.

6. As indicated by the haematoxylin test, the aluminum absorbed by the

plants accumulated in the cortex, mainly in the protoplasm, and was concentrated in the nuclei.

7. No accumulation of aluminum was detected by these tests in any other parts of the young plants, except in the cortex of the immersed roots.

REFERENCES

(1) Blair, A. W., and Prince, A. L. 1923 Studies on the toxic properties of soils. *Soil Sci.* 15: 109–129.

(2) Burgess, Paul S., and Pember, F. R. 1925 "Active" aluminum as a factor detrimental to crop production in many acid soils. R. I. Agr. Exp. Sta. Bul. 194.

(3) Hartwell, Burt L., and Pember, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soils on barley and rye. *Soil Sci.* 6: 259–279.

(4) Hoffer, J. N., and Carr, R. H. 1923 Accumulation of aluminum and iron compounds in corn plants and its probable relation to root rots. *Jour. Agr. Res.* 23: 810.

(5) Line, J. 1926 Aluminum and acid soils. *Jour. Agr. Sci.* 16: 335–364.

(6) McGeorge, W. T. 1925 The influence of aluminum, manganese, and iron salts upon the growth of sugar cane, and their relation to the infertility of acid island soils. Exp. Sta. of Hawaiian Sugar Planters' Assoc. Agr. and Chem. Ser. Bul. 49.

(7) Magistad, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181–226 (The earlier literature is there cited).

(8) Rothert, W. 1906 Das verhalten der pflanzen gegenuber dem aluminum. *Bot. Ztg.* 64: 7–52.

(9) Stoklasa, Jules 1911 Del importance physiologique du manganese et de l'aluminum dans la cellule vegetale. *Compt. Rend. Acad. Sci.* (Paris) 152: 1340–1342.

(10) Stoklasa, Jules 1925 Aluminum in organic life. *Internatl. Zeit. Agr.* 3: 3.

PLATE 1

Effect of Aluminum on Corn Roots

Fig. 1. The corn plant to the left was grown in a solution to which 28.8 p.p.m. of aluminum had been added during half of each week. The plant on the right had no aluminum added to the culture solution. Note the short, stubby roots of the aluminum-treated plant.

Fig. 2. Haematoxylin-stained cross section of a corn root from a plant grown in a solution to which 21.6 p.p.m. of aluminum had been added. The protoplasm of the cortex is stained, and the nuclei of the cortical cells are deeply stained. None of the cells of the stele are stained.

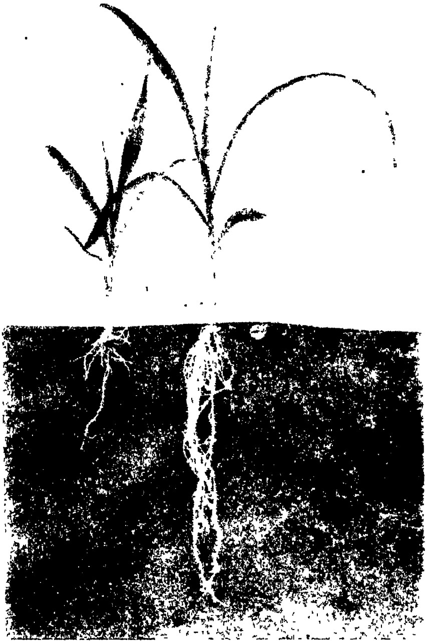

FIG. 1

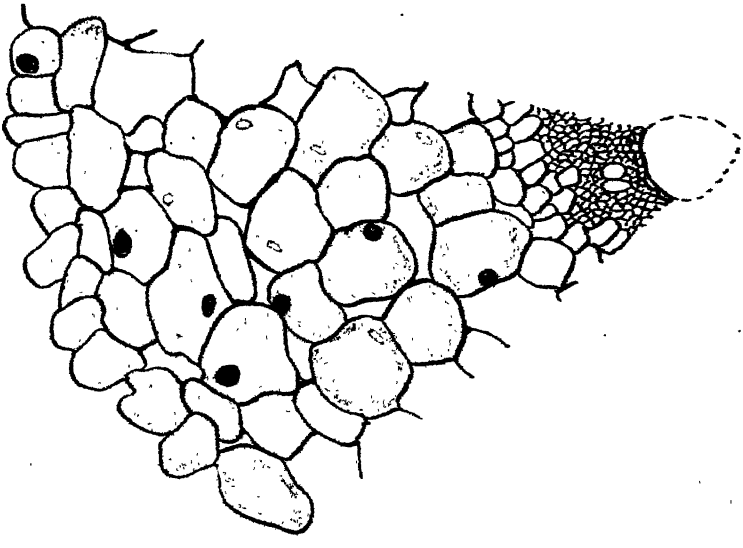

FIG. 2

NITRATE ACCUMULATION UNDER A MULCH

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Numerous investigations of the relation of organic matter to the accumulation of soil nitrates have been made. In most of those reported in the literature the organic matter was incorporated in the soil in some manner. Only two investigators have reported work of this kind in which organic matter was applied to the surface of the soil as a mulch. Scott (4), working with a silt loam at the Kansas station, found that 2 tons or more of straw applied to wheat as a mulch had a depressive influence on soil nitrates. Albrecht (2), (3) has reported a marked depressive influence of a straw mulch in an uncropped silt loam. The results here reported are of especial interest because the relation of a mulch to nitrate accumulation was found to be exactly the reverse of that reported by Scott and Albrecht. As will be seen, however, the conditions of the experiment were somewhat different from those heretofore reported.

DESCRIPTION OF SOIL AND CONDITIONS

The soil studied is a glacial till dervied from crystalline and sedimentary rocks and tentatively classified as Gloucester sandy loam. The topography is hilly and the plots studied are located on the eastern slope of a drumloid having a grade of approximately 15 per cent. Some physical and chemical characteristics of the soil are given in table 1. The climatic conditions prevailing were rather typical of those of the northeastern United States, the mean[2] rainfall at Amherst being 44.17 inches, and the mean temperature 46.8°F. Seasonal rainfall and temperature are given in table 3.

The plots have been used in orchard studies of the pomology department for several years, and are known as Block G-H of the orchard series. Figure 1 shows a plat of the experiment. From 16 to 20 apple and pear trees, including the varieties McIntosh, Wealthy, and Bosc (pear), planted in 1911, were growing on the plots. Each plot had an area of approximately one-fourth acre. No fertilizer was applied to any plot. A cover crop was sown on the cultivated plots each year about the middle of summer.

Since 1922 a mulch composed of waste hay and straw has been applied to the uncultivated plots in the spring and fall of each year, at the rate of 6 to 8 tons per acre: the fall application was omitted in 1926. The mulch was placed mainly under the overhanging branches of the trees and occupied about two-thirds the total area of the plots: it varied in depth from 2 to 12 inches with an average of 6 to 8 inches. Since the beginning of the mulching practice the mulch has not been removed nor the soil plowed. Thus, there has been a steady accumulation of decomposing organic matter, diminished only by that which is lost through the natural channels of decay and weathering. The accumulation of 1926, estimated at 8 to 10 tons to the acre, was so thick that weeds and grasses were almost wholly absent. An examination of the mulch at the latter part of the growing season showed that the whole mass was pretty well broken down with the lower layer well humified. Chemical analyses of the mulch material are given in table 2.

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[1] In coöperation with Dr. J. K. Shaw of the pomology department.

[2] Mean climatological data from Summary of Climatological Data for the United States by Weather Bureau, U. S. Dept. Agr.

TABLE 1

Physical and chemical properties of the soil

Mechanical analysis:	*per cen*
Fine gravel (2–1 mm.)	3.6
Coarse sand (1–0.5 mm.)	6.9
Medium sand (0.5–0.25 mm.)	10.0
Fine sand (0.25–0.10 mm.)	16.3
Very fine sand (0.10–0.05 mm.)	33.8
Silt (0.05–0.005 mm.)	19.7
Clay (0.005 mm.)	9.7
Loss on ignition:	*per cent*
Cultivated	5.9
Mulched	5.6
Hydrogen-ion concentration:	*pH*
Cultivated	6.2–6.4
Mulched	5.4–5.6
Lime absorption:*	*pounds CaO*
Cultivated	1115
Mulched	1784

* Jones' calcium acetate method used.

The conditions of the experiment here reported differ from those previously reported in respect to: 1) character of soil, 2) treatment of soil, 3) accumulation of mulch, 4) kind of mulch, 5) crop, and 6) climatic conditions. Furthermore, it should be noted that nitrate determinations were not made in this experiment until 1925, three years after the mulch was first applied.

METHODS

Nitrate determinations were made on composite soil samples drawn at weekly intervals during the active growing seasons or at other intervals as indicated later, during 1925 and 1926. In sampling, the mulch was pushed away and borings were drawn with an auger (diameter 1.5 inch) to a depth of 7 inches. Temperature and moisture conditions were also determined. The soil samples were immediately taken to the laboratory and nitrates determined in the moist soil by the phenol disulfonic acid method.

TABLE 2

Chemical analyses of the mulch material

	per cent
Total nitrogen of fresh mulch	1.15*
Total nitrogen of old mulch:	
Upper layer	2.28*
Lower layer	1.57*
Humus‡ content of fresh mulch	3.21†
Humus content of old mulch:	
Upper layer	8.6†
Lower layer	7.9†

* Moisture-free basis. Nitrate nitrogen not included.
† Air-dry basis.
‡ Soluble in 4 per cent ammonia solution.

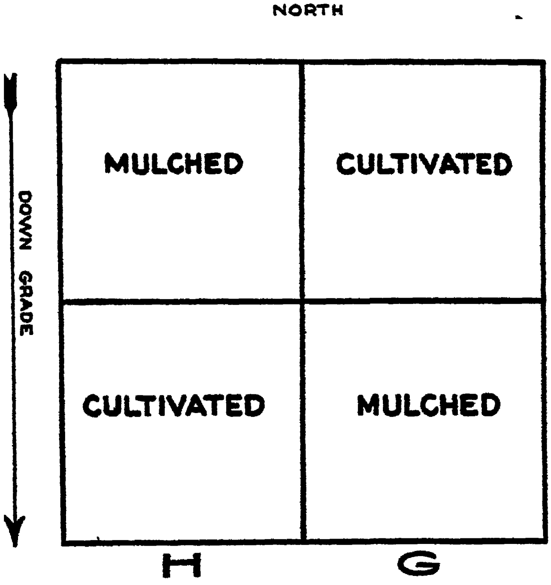

FIG. 1. PLAT OF THE EXPERIMENT

RESULTS

The data on nitrates, moisture, temperature, and rainfall are presented in tables 3 to 8.

TABLE 3

Mean seasonal temperature and precipitation† at Amherst, Massachusetts*

MONTH	TEMPERATURE	PRECIPITATION
	°F.	inches
January	23.8	3.38
February	22.3	3.27
March	34.5	3.60
April	45.7	3.18
May	57.0	3.80
June	64.6	3.61
July	71.0	4.40
August	68.4	4.36
September	61.4	3.66
October	51.5	3.71
November	38.3	3.62
December	22.8	3.58
Annual	46.8	44.17

* For 84 years.
† For 20 years.

TABLE 4

Soil temperature

1925					1926				
DATE	Cultivated		Mulched		DATE	Cultivated		Mulched	
	G	H	G	H		G	H	G	H
	°C	°C	°C	°C		°C	°C	°C	°C
5/8	8	9	8	9	5/24	12	12	12	12
15	11	12	11	12	6/1	15	15	13	13
22	13	13	13	13	8	13	13	12	12
29	12	12	12	12	15	16	16	14	14
6/5	18	18	18	18	22	15	15	13	13
12	16	17	14	14	29	16	16	15	15
19	18	18	16	15	7/6	17	17	15	15
26	15	15	14	14	13	17	17	16	16
7/3	16	16	16	16	20	18	18	17	17
10	19	19	17	18	27	18	18	17	17
17	20	20	18	18	8/3	21	22	19	19
24	18	18	17	18	10	19	20	18	18
31	17	17	17	17	17	18	16	18	19
8/7	18	18	19	19	24	18	18	17	17
14	18	18	18	18	31	16	15	16	16
21	18	18	18	17	9/7	16	16	16	16
28	16	16	16	17	14	14	12	14	14
9/4	17	17	17	17	21	15	15	16	16
25	13	13	15	14	28	13	12	12	13
10/16	11	11	11	12	10/5	10	10	11	10
					19	8	7	10	10
					11/2	6	6	8	8
					16	11	10	9	9
					12/2	3	4	6	6
					14	....	....	[3]	[3]
					30	....	....	[3]	[3]
					1/15	....	....	[2]	[2]
Average	15.6	15.8	15.3	15.4		14.4	14.2	13.9	14.0

The difference in the nitrate content of the soils under mulch and cultivation is marked. In contrast with the results of Albrecht, it is striking. At no time within the periods studied did the average nitrate content of the cultivated plots reach that of the mulched plots, although at three times in 1925 the curves for the two almost converged. This occurred within the most active growing period when the greatest draft was made on nitrates. In both years the divergence of the nitrate curves is greatest during the latter part of the season. The figures for 1926 run unusually high for field soils. That such concentrations and even greater ones are not impossible when very favorable conditions for nitrification exist is proved by the results of others (1), (4), (5). It appears that such favorable conditions existed on Block G-H in 1926.

TABLE 5

Nitrates, Block G-H 1925

Dry weight basis

DATE	CULTIVATED			MULCHED		
	G	H	Average	G	H	Average
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
5/8	Tr.	11.6	5.8	29.1	13.1	21.1
15	5.9	11.8	8.9	18.3	6.2	12.3
22	3.0	7.2	5.1	10.4	14.0	12.2
29	4.3	17.7	11.0	15.8	9.6	12.7
6/5	3.7	18.7	11.2	28.4	14.4	21.4
12	4.2	11.6	7.9	24.6	60.5	42.6
19	7.3	35.3	21.3	36.5	41.9	39.2
26	14.9	36.2	25.6	32.9	34.6	33.8
7/3	9.4	26.6	18.0	21.2	18.0	19.6
10	5.1	21.4	13.3	19.7	23.5	21.6
17	7.3	28.4	17.9	48.8	40.0	44.4
24	4.4	9.4	6.9	25.4	32.5	29.0
31	4.4	6.0	5.2	23.6	18.4	21.0
8/7	11.1	10.9	11.0	49.9	95.5	72.7
14	3.1	21.0	12.1	67.0	34.8	50.9
21	8.1	16.7	12.4	40.9	56.0	48.5
28	1.3	5.7	3.5	34.9	66.2	50.6
9/4	1.4	5.5	3.5	96.6	81.1	88.9
25	1.5	1.4	1.5	63.4	53.2	58.3
10/16	Tr.	1.3	0.7	38.1	77.5	57.8
Average.....	5.6	15.2	10.1	36.3	39.6	37.9

When the factors concerned are examined as to the probable explanation of these results in view of the results of others, we find the following: First, the soil used was light, loose, well drained, and assumably well aerated. In these respects the conditions were noticeably different from those of Albrecht. As shown by him and others, aeration has a beneficial influence on nitrification in soils. Second, the mulch material was allowed to accumulate and decompose until there was a large amount of readily nitrifiable material present. The original mulch material was probably somewhat richer in nitrogen than the straw used by others. With a rather constant and large supply of humified and, to a certain extent, soluble nitrogenous compounds intense nitrification and accumulation of nitrates may be expected. A study of the chemical composition of the mulch material with respect to its nitrogen and humus content (table 2) suggests the following probable changes: because of the action of enzymes or even unassociated oxidation the carbohydrates of the fresh mulch were considerably reduced in quantity, next the proteins were broken down into soluble hydrolytic products which were leached into the soil and readily nitrified. Since there was no large supply of cellulose in the soil there was no cause for activity of denitrifying organisms: with the protection of a heavy mulch leaching was reduced. Hence, conditions were favorable to nitrate accumulation.

TABLE 6

Moisture percentage, Block G-H, 1925

Dry weight basis

DATE	CULTIVATED			MULCHED		
	G	H	Average	G	H	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5/8	26.4	28.3	27.4	27.9	27.8	27.9
15	26.5	29.7	28.1	29.0	31.1	30.1
22	24.8	24.2	24.5	28.8	27.1	28.0
29	23.6	27.6	25.6	29.9	27.7	28.8
6/5	22.9	27.9	25.4	28.3	28.3	28.3
12	18.2	21.4	19.8	25.6	27.4	26.5
19	18.4	19.8	19.1	26.3	24.2	25.3
26	23.5	28.5	26.0	30.5	25.6	28.1
7/3	22.3	27.6	25.0	28.3	27.0	27.7
10	23.5	28.2	20.9	30.6	27.0	28.8
17	22.9	28.1	25.5	29.7	29.2	29.5
24	27.2	31.2	29.2	31.9	29.4	30.7
31	26.7	28.2	27.5	32.5	28.0	30.3
8/7	26.3	30.9	28.6	31.2	28.9	30.1
14	27.0	31.8	29.4	29.2	30.0	29.6
21	20.0	25.5	22.8	24.7	22.8	23.8
28	18.0	25.4	21.7	23.5	22.5	23.0
9/4	22.6	26.3	24.5	30.0	29.0	29.5
25	18.6	26.0	22.3	24.7	23.4	24.1
10/16	21.4	28.4	24.9	28.5	28.7	28.6
Average.....	23.0	27.3	24.9	28.5	27.2	27.9

What the situation was in the early period of the experiment with respect to nitrates, is not known, inasmuch as no determinations were made at that time. With other workers, we have found that the action of the mulch was to

TABLE 7

Nitrates, Block G-H 1926

Dry weight basis

DATE	CULTIVATED			MULCHED		
	G	H	Average	G	H	Average
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
5/10	3.3	8.5	5.9	32.7	23.8	28.3
17	4.4	6.4	5.4	35.6	35.6	35.6
24	4.3	8.1	6.2	69.9	67.0	68.5
6/1	3.3	3.6	3.4	91.0	54.6	72.8
8	3.5	3.7	3.6	97.5	93.5	95.5
15	3.3	41.5	22.4	73.2	44.3	58.8
22	5.4	13.1	9.3	88.3	92.5	90.4
29	17.0	30.5	23.8	133.0	160.0	146.5
7/6	79.2	87.0	83.1	378.0	527.0	452.5
13	38.8	103.0	70.9	265.0	387.0	326.0
20	14.5	47.2	30.9	205.0	87.0	146.0
27	19.0	34.3	26.7	205.0	87.5	146.3
8/3	14.7	46.5	30.6	171.0	51.0	111.0
10	12.7	34.5	23.6	267.0	62.2	164.6
17	3.1	3.2	3.2	36.6	58.1	47.4
24	Tr.	13.6	6.8	177.0	64.9	121.0
31	10.4	15.7	13.1	160.0	108.0	134.0
9/7	Tr.	18.5	9.3	111.0	132.0	121.5
14	Tr.	Tr.	Tr.	65.8	111.0	88.4
21	Tr.	Tr.	Tr.	171.0	169.0	170.0
28	Tr.	Tr.	Tr.	210.9	159.0	185.0
10/5	Tr.	23.1	11.6	157.0	101.0	129.0
19	Tr.	2.9	1.5	320.0	249.0	284.5
11/2	Tr.	Tr.	Tr.	156.8	162.3	159.6
17	Tr.	Tr.	Tr.	.....	169.0	84.5
12/2	....	....	....	104.0	74.6	89.3
14	....	....	....	109.3	30.4	69.9
30	....	....	....	65.9	56.0	61.0
1/15	....	....	....	7.0	11.5	9.7
Average.....	14.8	27.3	19.6	141.2	118.2	127.5

TABLE 8

Moisture percentage, Block G-H 1926

Dry weight basis

DATE	CULTIVATED			MULCHED		
	G	H	Average	G	H	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5/10	20.4	33.9	27.2	32.9	29.4	31.2
17	30.7	34.5	32.6	33.3	30.9	32.1
24	25.7	30.2	28.0	30.5	28.2	29.4
6/1	25.7	30.9	28.3	35.0	29.0	32.0
8	22.9	29.4	26.2	30.5	24.5	27.5
15	25.5	32.9	29.2	40.9	30.8	35.9
22	16.5	25.6	21.1	27.4	25.0	26.2
29	18.9	26.8	22.9	28.8	24.3	26.6
7/6	18.8	23.2	21.0	27.2	25.4	26.3
13	18.1	23.7	20.9	27.0	21.7	24.4
20	24.0	25.6	24.8	30.6	24.7	27.7
27	22.4	19.6	21.0	19.7	17.0	18.4
8/3	25.5	28.7	27.1	35.3	29.8	32.6
10	14.1	19.2	16.7	28.1	22.1	25.1
17	23.4	28.4	25.9	34.1	36.9	35.5
24	18.3	24.5	21.4	28.8	24.1	26.5
31	16.0	21.5	18.8	31.6	25.2	28.4
9/7	23.1	30.7	26.9	31.8	26.5	29.2
14	15.0	18.7	16.9	26.3	23.5	24.9
21	11.6	17.6	14.6	28.2	22.9	25.6
28	17.9	21.4	19.7	34.1	26.3	30.2
10/5	12.8	19.8	16.3	25.6	21.3	23.5
19	25.2	25.6	25.4	26.3	22.9	24.6
11/2	25.8	30.2	28.0	28.4	29.9	29.2
17	25.4	29.9	27.7	30.3	25.0	27.7
12/2	30.0	32.4	31.2	34.5	30.2	32.4
14	....	....	....	26.6	21.7	24.2
30	....	....	....	32.7	26.7	29.7
1/15	....	....	....	20.5	27.4	24.0
Average.....	21.3	26.3	23.8	29.9	25.94	27.96

conserve moisture and stabilize temperature. The action of these factors, however, was not unfavorable toward nitrification.

CONCLUSION

Under soil, climatic, and mulching conditions such as those described above, a mulch is much more effective than cultivation in causing nitrate accumulation.

REFERENCES

(1) AALTONEN, V. T. 1926 Über die Umsetzungen der Stickstoffsuerbindungen im Waldboden. Comm. ex inst. quaest. forest. finl. ed. 10. Helsingfors.

(2) ALBRECHT, W. A. 1922 Nitrate accumulation under straw mulch. *Soil Sci.* 14: 299–305.

(3) ALBRECHT, W. A., AND UHLAND, R. E. 1925 Nitrate accumulation under the straw mulch. *Soil Sci.* 20: 253–265.

(4) SCOTT, H. 1921 The influence of wheat straw on the accumulation of nitrates in the soil. *Jour. Amer. Soc. Agron.* 13: 233–258.

(5) STEPHENSON, R. E. 1921 The effect of organic matter on soil reaction. II. *Soil Sci.* 12: 145–162.

NITRIFICATION IN SOILS: II

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INTRODUCTION

In a previous paper (1) the author has discussed the results of the nitrification of mono-amino-mono-carboxylic acids and tryptophan in a particular soil. In this paper an attempt is made to show the nitrifiability of the amino acids of the remaining series, that is, mono-amino-dicarboxylic acids, di-amino-mono-carboxylic acids and hetero-cyclic ones along with other organic compounds of nitrogen in the same medium.

EXPERIMENTAL

The following compounds were used in these series of experiments: Nicotine, brucine, aspartic acid, asparagine, uric acid, hippuric acid, histidine dichloride, arginine, strychnine, glutamic acid hydrochloride, xanthine, and lysine. The first six were used in the first series of experiments and the remaining in the next. Ammonium sulfate was also used in both series along with the control. As most of these substances were not near at hand and had to be sent for from foreign countries, the amino acids belonging to the same series could not be tried together but were utilized when they were available.

Before they were used their purity was determined by estimating their total nitrogen by the Kjeldahl method. During the estimation of nitrogen some difficulty was encountered. As usual, each of the compounds was first digested for three hours with the sulfuric acid and sulfates of potassium and copper and then distilled with NaOH solution into $0.1N$ sulfuric acid. The compounds having chain formulas only, gave results similar to the theoretical quantities; but the nitrogen in the ring compounds resisted the action of the strong acid and allowed itself to be acted upon only partially, giving low results. Then the modified Kjeldahl method with mercuric oxide (4) was tried. The figures obtained were similarly low. After this the hours of digestion by the Kjeldahl method were gradually prolonged up to 24. The results given in table 1, show that by prolonging the hours of digestion, nearly all the ring nitrogen can be converted into ammonium salts.

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[1] The author takes this opportunity of expressing his thanks to Mr. G. Clarke, Director of Agriculture, United Provinces, for his valuable suggestions, and to Messrs. G. R. Saksena, and L. S. Nigam for rendering help in laboratory work.

The quantities of nitrogen found in the compounds used, together with those calculated from the formulas, are given in table 2. All the calculations in these experiments are based upon the actual quantities of nitrogen found and not upon the theoretical ones as in the previous paper (1). The lysine was prepared

TABLE 1

Quantities of nitrogen determined by the Kjeldahl method after different hours of digestion

NAME OF AMINO ACID	TIME OF DIGESTION	NITROGEN BY FORMULA	NITROGEN BY KJELDAHL'S METHOD	NITROGEN BY MERCURIC OXIDE METHOD
	hours	*per cent*	*per cent*	*per cent*
1. Histidine dichloride	3	18.42	16.94	15.59
	15		17.69	
	24		18.01	
2. Xanthine	3	36.84	35.51	
	13		35.42	27.86
	24		36.22	
3. Nicotine	3	17.28	16.85	13.77
	13		16.24	
	24		17.03	
4. Brucine	3	7.11	7.00	
	13		6.52	5.60
	24		7.05	
5. Uric acid	3	33.33	31.83	28.35

TABLE 2

Results of nitrogen determinations

NUMBER	NAME OF COMPOUND	NITROGEN BY FORMULA	NITROGEN BY KJELDAHL'S METHOD
		per cent	*per cent*
1	Ammonium sulfate	21.21	21.16
2	Arginine	29.17	28.78
3	Asparagine	21.21	20.53
4	Aspartic acid	10.53	10.39
5	Brucine	7.11	7.05
6	Glutamic acid hydrochloride	7.71	7.56
7	Hippuric acid	7.82	7.33
8	Histidine dichloride	18.42	18.01
9	Lysine	19.18	.....
10	Nicotine	17.28	17.03
11	Strychnine	8.38	8.14
12	Uric acid	33.33	33.04
13	Xanthine	36.84	36.22

in the form of a solution from lysine picrate (3) and the lysine content of the solution was determined by estimating its nitrogen by the Kjeldahl method.

The soil used in this work was the same as that employed in the previous experiments, because a very large sample of the surface soil from a plot of the estate of the Agricultural College, Cawnpur, was secured in the very beginning of the experiments, so that the whole of the work could be done in the same medium and to avoid a change in the soil conditions. The soil was a light loam and its chemical and mechanical compositions and its pH value were described in the first paper (1).

For a description of the background and methods of the experiments here reported, the reader is referred to the preceding work (1). The solutions of each compound containing nearly 15 mgm. of nitrogen were used after neutralization with an acid or alkaline solution as the case was. At the end of each incubation period instead of the whole of the soil being transferred from the jar on to the Buchner filter for leaching out nitrate content as before, 10 gm. of it was weighed out from the jar for a study of the variations of pH values and the rest was utilized on the Buchner filter for the estimation of nitric nitrogen. The nitrates were always calculated back to the original 100 gm. of the soil used, and recorded as milligrams of nitric nitrogen.

DISCUSSION OF RESULTS

The results of the first series of experiments are given in table 3 and those of the second in table 4. In the previous paper the nitrifiability of mono-amino-mono-carboxylic acids and that of tryptophan was discussed. The results showed that, leaving aside special cases, the acids belonging to the mono-amino-mono-carboxylic acid series were nitrified at about the same rate. Now the nitrifiability of the amino acids of the remaining series and that of other nitrogenous organic compounds mostly found in the soil (6,7,8) is to be considered.

After the first series of mono-amino-mono-carboxylic acids comes the series of mono-amino-dicarboxylic acids. Of the latter two acids, aspartic and glutamic acids with formulas $HOOC \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH$ and $HOOC \cdot CH_2 \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH$ respectively, were used in these investigations. The results of the nitrification of aspartic acid are given in table 3 and that of glutamic acid in table 4. For the sake of comparison it would have been better had both the acids been nitrified simultaneously, but as stated before they were used in separate series on account of their arrival at different times. Their figures of nitrification show that after the incubation period of 30 days the maximum and the minimum limits of nitrification of both acids are the same, that is, about 57 and 43 per cent. During the 40 days' incubation the figures of glutamic acid have always remained between the above limits, but those of aspartic acid have exceeded the above maximum.

A comparison of the nitrifiability of mono-amino-mono-carboxylic acids with that of mono-amino-dicarboxylic acids shows that the members of the mono-

TABLE 3

Results of the nitrification of the first series of amino acids

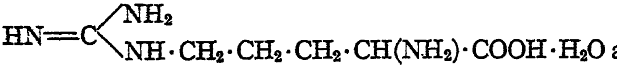
DATE	CONTROL	NICOTINE			BRUCINE			ASPARTIC ACID			ASPARAGINE			HIPPURIC ACID			AMMONIUM SULFATE		
	Nitric nitrogen found at the end of incubation period	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Nitrification of amino acids in 30 days																			
2/16/25–3/18/25	5.34	15.08	9.74	61.92	5.28	−0.06	....	13.29	7.95	53.00	14.00	8.66	57.73	10.10	4.76	31.73	16.15	10.81	70.65
4/ 7/25–5/ 8/25	7.54	20.07	12.53	79.66	....	....	....	15.22	7.68	51.20	15.42	7.88	58.53	12.95	5.41	36.07	20.56	13.02	85.10
								Uric acid											
5/25/25–6/23/25	6.51	18.72	12.21	77.62	7.95	1.44	8.88	12.96	6.45	43.00	16.65	10.14	66.45	14.96	8.45	56.33	18.47	11.96	78.17
7/21/25–8/19/25	5.96	15.17	9.21	58.55	7.39	1.43	8.82	14.60	8.64	57.60	14.97	9.01	59.04	12.56	6.60	44.00	17.78	11.82	77.25
Nitrification of amino acids in 40 days																			
2/16/25–3/28/25	6.06	16.29	10.23	65.03	6.03	−0.03	....	....	....	....	16.01	9.95	66.33	11.39	5.33	35.53	17.41	11.35	74.18
4/ 7/25–5/17/25	9.56	17.87	8.31	52.83	10.28	0.72	4.43	18.21	8.65	57.67	16.72	7.16	47.73	16.87	7.31	48.73	18.76	9.20	60.13
								Uric acid											
5/25/25–7/ 3/25	6.58	16.61	10.03	63.76	9.05	2.47	15.23	15.28	8.70	58.00	15.58	9.00	58.98	13.52	6.94	46.27	18.41	11.83	77.32
7/21/25–8/29/25	6.85	17.37	10.52	66.88	6.97	0.12	0.74	....	....	....	16.25	9.40	61.60	11.75	4.90	32.67	18.52	11.67	76.27
Average...........................				65.78			6.38			53.41			57.58 61.52			41.42			74.87

TABLE 4

Results of the nitrification of the second series of amino acids

DATE	CONTROL	HISTIDINE DICHLORIDE			ARGININE			STRYCHNINE			GLUTAMIC ACID HYDROCHLORIDE			XANTHINE			LYSINE			AMMONIUM SULFATE		
	Nitric nitrogen found at the end of incubation period	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found	Nitric nitrogen found	Increase over the control	Nitrogen nitrified found
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Nitrification of amino acids in 30 days																						
10/ 9/25–11/ 8/25	6.43	15.92	9.49	62.15	16.21	9.78	65.20	5.32	−1.11	....	15.03	8.60	57.33	15.71	9.28	60.69	15.93	9.50	63.33	16.19	9.76	63.79
10/26/25–11/25/25	5.80	14.71	8.91	58.35	15.96	10.16	67.73	5.94	0.14	0.93	13.29	7.49	49.93	14.39	8.59	56.18	14.66	8.86	59.67	15.40	9.60	62.74
11/14/25–12/14/25	5.13	13.21	8.08	52.91	14.33	9.20	61.33	5.44	−0.69	....	11.61	6.48	43.20	13.80	8.67	56.70	12.85	7.72	51.47	15.17	10.04	65.62
1/29/26– 2/27/26	6.32	14.67	8.35	54.68	14.51	8.19	54.60	6.03	−0.29	....	13.67	7.35	49.00	14.60	8.14	53.24	13.39	7.07	47.13	.....	.....	.....
Nitrification of amino acids in 40 days																						
10/ 9/25–11/18/25	6.69	15.09	8.40	55.01	15.88	9.19	61.27	6.24	−0.45	....	14.66	7.97	53.13	16.54	9.85	64.42	13.55	6.86	45.73	17.03	10.34	67.58
10/26/25–12/ 5/25	5.67	13.56	7.89	51.67	15.53	9.86	65.73	6.04	0.37	2.47	13.47	7.80	52.00	15.24	9.57	62.59	13.76	8.09	53.93	16.54	10.87	71.04
11/14/25–12/24/25	5.33	13.43	8.10	53.04	14.62	9.29	61.93	5.46	0.13	0.87	12.07	6.74	44.93	13.92	8.59	56.18	12.26	6.93	46.20	15.20	9.87	64.51
1/29/26– 3/ 7/26	5.78	14.59	8.81	57.69	14.70	8.92	59.47	6.96	1.18	7.87	13.89	8.11	54.07	14.25	8.47	55.39	13.99	8.21	54.73	.....	.....	.....
Average........................				55.69			62.16			3.03			50.45			58.42			52.77			65.88

amino-dicarboxylic acid series are slightly more nitrifiable than those of the mono-amino-mono-carboxylic acid series; because the figures obtained from the latter series were generally below 50 per cent, whereas those obtained from the former were in a majority of cases over 50 per cent.

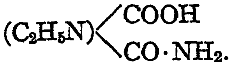
In the investigations of the third series of amino acids containing di-amino-mono-carboxylic acids, which are also called hexone bases, arginine  $HN{=}C\begin{array}{l}\diagup NH_2\\ \diagdown NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH\cdot H_2O\end{array}$ and lysine $H_2N\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$ were used. The nitrification of arginine differs a great deal from that of lysine. The former gave figures mostly above 60 per cent and never below 54; whereas the latter only once reached above 60 and had also shown figures below 50 per cent. This shows that there is a greater accumulation of nitric nitrogen from arginine than from lysine. It is probably due to the presence of guanidine group in arginine, which is readily saponifiable to ammonia and carbon dioxide and thereby brings about a rapid accumulation of nitrates.

To compare the nitrifiability of di-amino-mono-carboxylic acids with that of mono-amino dicarboxylic acids, the figures obtained undoubtedly show that arginine is superior to aspartic and glutamic acids in nitrifiability. In the case of lysine, the formation of nitric nitrogen has fluctuated between 63 and 45 per cent and in that of mono amino-dicarboxylic acids, between 63 and 43 per cent. Though the maximum is the same in both cases yet the minimum of lysine indicates that it is a little better nitrifiable substance than the mono-amino-dicarboxylic acids. The data, therefore, give indications of the superiority of diamino-mono-carboxylic acids to mono-amino-dicarboxylic acids and mono-carboxylic acids in nitrifiability in the soil.

The fourth series of amino acids consists of heterocyclic ones, of which tryptophan was used in the previous experiments and histidine in the present. In the majority of determinations the percentage of nitrogen nitrified in both the cases has ranged between 50 and 60, histidine crossing once the maximum and tryptophan once the minimum. Thus there appears a similarity in the nitrification of the two compounds, though it seems possible for histidine to show at times better nitrifiability than tryptophan.

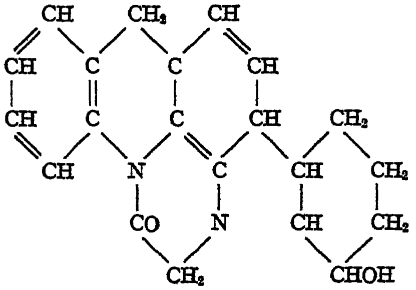
In the former paper (1) while studying the nitrifiability of tryptophan and mono-amino-mono-carboxylic acids it was remarked that the compound containing both ring and chain nitrogen has better nitrification value than the compounds having chain nitrogen only. This remark appears to hold good when the comparison is made only between heterocyclic compounds and mono-amino-mono-carboxylic acids; because it is seen from the figures given in table 4 that the nitrification values of the compounds like lysine and arginine, which also have only chain nitrogen, are not in any way inferior to those of tryptophan and histidine having both chain and ring nitrogen.

Although the comparative availability of the members of different series of amino acids has been judged, it can best be done when the average of the figures obtained for each are taken into consideration. This will be taken up later after the results of the nitrification of the following other nitrogenous compounds used in these investigations are discussed: asparagine, hippuric acid, uric acid, nicotine, brucine, strychnine, and xanthine.

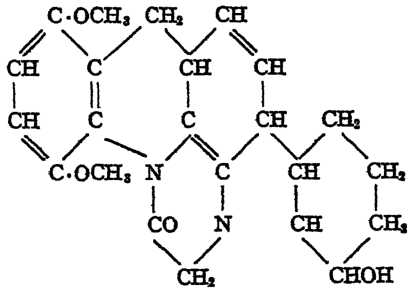
Asparagine is the monoamide of aspartic acid with the formula  $(C_2H_5N)\begin{cases}COOH\\CO\cdot NH_2\end{cases}$. On account of its being available in very small quantity it was tried only twice and not four times as in the case of other compounds. Its quantity being exhausted and to fill up the gap in the series, uric acid was used in its place, the results of which will be discussed with those of xanthine. The nitrifiability of asparagine has varied from 57 to 58 per cent during the incubation of 30 days, and, from 47 to 66 per cent during that of 40 days, but the average of the two incubations comes to about 58 and 57 per cent respectively. It is interesting to note here that asparagine has shown better nitrifiability than aspartic acid. This is probably due to the presence of an amide group in the substance.

Hippuric acid is benzoyl glycine with the formula $C_6H_5CO\cdot NHCH_2\cdot COOH$ and therefore is a derivative of the mono-amino-mono-carboxylic acid series. Generally the nitrification of hippuric acid is lower than that of any compound thus far considered in this paper but it appears very similar to that of mono-amino-mono-carboxylic acids as can be judged from the figures obtained from them.

Strychnine

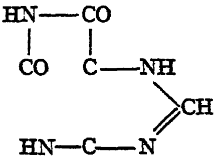


and brucine

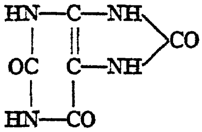


are the derivatives of quinoline. The fact that both of them are highly toxic must have produced in the very beginning some fatal effect upon the micro-organisms of the soil and therefore not only have shown least nitrification values but have also given negative results. Brucine is much less toxic than strychnine and has consequently almost always given positive results; whereas strychnine has for the greatest part given negative results during the incubation of 30 days and positive during that of 40 days, but far less than those of brucine. Further, if the figures obtained for both these poisonous compounds during the different periods of incubation are followed, it is noticeable that when the compounds are mixed with the soil their toxicity gradually decreases and is completely destroyed if kept there for a sufficient length of time; because both of them have mostly given positive results and sometimes increased production of nitric nitrogen during the incubation period of 40 days. From this it is to be inferred that the toxic effect of such poisonous compounds in the soil is only a temporary one and that the phenomenon of nitrification again makes its appearance when the toxic effect is removed. This may possibly be due to the very gradual decomposition or transformation of these poisonous compounds into harmless ones.

Of the remaining compounds used, xanthine

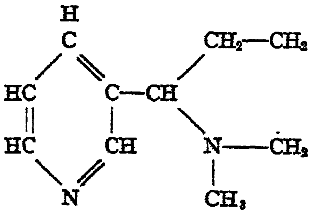


and uric acid



belong to the group called purine derivatives. The nitrifiability of xanthine has varied from 53 to 64 per cent, and that of uric acid from 58 to 66 per cent. Both of them have shown better nitrification values than any of the compounds hitherto considered except arginine. This may probably be due to their being more easily decomposed into urea, ammonia, and other products.

The case of nicotine



is a peculiar one. Though it is highly toxic and poisonous like strychnine and brucine, still it has shown the greatest nitrifiability, excelling all the other compounds used in these investigations and approaching very near to ammonium sulfate. The nitrification of its nitrogen has fluctuated from 52 to 79 per cent. This may be due to some of the special properties and the composition of the compound. Because nicotine is most readily oxidized and on account of the presence in its molecule of the pyrolidine group, which is not so stable as the six atom ring, it is very likely that it undergoes decomposition and oxidation very easily in the soil, producing ammonia and carbon dioxide.

Now turning to ammonium sulfate it appears from the data given in this as well as in the previous paper (1) that none of the nitrogenous organic compounds can compete with it in nitrifiability simply because the nitrogen of ammonia is directly nitrified, whereas the organic nitrogen has first to pass through the decomposition process to be converted into ammonia and then into nitrate.

In the previous paper (1) the comparison of the nitrifiability of the ring and the chain nitrogen atoms was postponed until furthur data on the point were collected. In order to do this it seemed essential to take into account on the one hand, compounds having ring nitrogen and on the other compounds having only chain nitrogen but both containing the same number of N atoms. Thus out of the substances used in the experiments the compounds lysine ($C_6H_{14}N_2O_2$) and asparagine ($C_4H_8N_2O_3$) of the chain formula and, tryptophan ($C_{11}H_{12}N_2O_2$), nicotine ($C_{10}H_{14}N_2$), brucine ($C_{23}H_{26}N_2O_4$) and strychnine ($C_{21}H_{22}N_2O_2$) of the ring formula contain two atoms of nitrogen. The average percentage of the nitrogen nitrified in each case is thus: lysine 52.77, asparagine 57.58, nicotine 65.78, brucine 6.38, strychnine 3.03 and tryptophan 51.99. Of the four compounds of the ring formula, three have shown less nitrification than the two compounds of the chain formula. Thus in the majority of cases the compounds containing ring nitrogen have produced less nitrate than those containing the chain nitrogen. Again, leaving aside the special cases of nicotine, brucine, and strychnine, the figures of the remaining compounds show that there is a depression of nitrification, though slight in the case of tryptophan.

Further the compounds, arginine ($C_6H_{14}N_4O_2$) of the chain formula and uric acid ($C_5H_4N_4O_3$) and xanthine ($C_5H_4N_4O_2$) of the ring formula, contain four atoms of nitrogen. The average nitrifiability of their nitrogen is thus: arginine 62.16, uric acid 61.52, and xanthine 58.17. These results also indicate that there is less production of nitric acid from the ring nitrogen than from the chain one.

Thus it appears that the ring nitrogen is not only slowly and difficultly acted upon by strong acids as pointed out before when determining total nitrogen by the Kjeldahl method but also resists the action of the microörganisms of the soil to a greater extent than the chain nitrogen.

From the discussion of the results it is observed that the nitrification of pure nitrogenous organic compounds is in some measure correlated to their compositions and properties. This becomes clearer if the compounds used and their average nitrifiability are considered according to their chemical classification as given in table 5. The average is calculated from all the figures obtained for each compound. The negative results are eliminated from the averages and are interpreted merely as representing the absence of nitrification. The maximum and the minimum represent the greatest and the least average percentage of nitrogen nitrified of any two compounds belonging to the same series.

TABLE 5

The maximum and the minimum of the average percentage of nitrogen nitrified of any two compounds in each group

NUMBER	NAME OF GROUP	NAME OF COMPOUND	NITROGEN NITRIFIED AVERAGE—MAXIMUM	NAME OF COMPOUND	NITROGEN NITRIFIED AVERAGE—MINIMUM
			per cent		*per cent*
1	Mono-amino-mono-carboxylic acid series	Phenylalanine	47.08	Tyrosine*	36.67
2	Mono-amino-dicarboxylic acid series	Aspartic acid	53.41	Glutamic acid hydrochloride	50.45
3	Diamino-mono-carboxylic acid series	Arginine	62.16	Lysine	52.77
4	Heterocyclic amino acid series	Histidine dichloride	55.69	Tryptophan	51.99
5	Purine derivatives	Uric acid	61.52	Xanthine	58.42
6	Quinoline derivatives	Brucine	6.38	Strychnine	3.03
7	Pyridine derivatives	Nicotine	65.78	....	.....

* *The average figures of tyrosine are lower in the above table than those of table 7 of the previous paper (1) on account of a miscount at that time in calculating the average, not from eight figures as usual, but from only four figures of the 30 days' period. Accordingly the graded list of various amino-acids made out of preceding investigations requires slight alteration, the tyrosine is to be placed last and not last but one.*

Thus it becomes evident from the maximum as well as from the minimum columns that quinoline derivatives are the least nitrified and the next are the mono-amino-mono-carboxylic acids. The mono-amino-dicarboxylic acids have shown better nitrification values than the above two classes of compounds. The diamino-mono-carboxylic acids have given still higher results, higher even than heterocyclic amino acids, which are only slightly better than the dicarboxylic acids. Leaving aside the special case of nicotine, which is a pyridine derivative and which has given the highest figures, the purine derivatives have also proved themselves to be the most readily nitrifiable substances, standing third in the maximum and first in the minimum. Thus there is marked difference among the nitrification values of each class of compounds, which is probably due to the difference in their composition and properties. Therefore the formation and accumulation of nitrates in the soil depends largely upon the nature of organic matter present or added therein.

TABLE 6

The percentage of nitrogen in the molecule of the compound and the average figures of nitrogen nitrified

NAME OF COMPOUND	NITROGEN BY FORMULA	NITROGEN NITRIFIED
	per cent	*per cent*
Xanthine	36.84	58.17
Uric acid	33.33	61.52
Arginine	29.17	62.16
Asparagine	21.21	37.58
Lysine	19.18	52.77
Histidine dichloride	18.42	55.69
Nicotine	17.28	65.78
Aspartic acid	10.53	53.41
Strychnine	8.38	3.03
Glutamic acid hydrochloride	7.53	50.45
Hippuric acid	7.82	41.42
Brucine	7.18	6.38

TABLE 7

Groups of compounds according to the percentage of nitrogen in their molecules and the average percentage of their nitrogen nitrified

GROUP	NITROGEN IN THE MOLECULES	AVERAGE PROPORTION OF NITROGEN NITRIFIED
	per cent	*per cent*
Group I:		
Xanthine	36.84	59.86
Uric acid	33.33	
Arginine	29.17	
Asparagine	21.21	
Group II:		
Lysine	19.18	56.91
Histidine dichloride	18.42	
Nicotine	17.28	
Aspartic acid	10.53	
Group III:		
Strychnine	8.38	25.32
Hippuric acid	7.82	
Glutamic acid hydrochloride	7.53	
Brucine	7.18	

Waksman (9) when studying the influence of microörganisms upon the carbon-nitrogen ratio in the soil, states that the amounts of nitrogen recovered as nitrates, were proportional to the percentage of nitrogen in the organic substances added. The results expressed in table 6 add considerable support to the above statement. The irregularities can be minimized if the compounds mentioned in the table are divided into groups; the first group to contain compounds having from 36 to 20 per cent nitrogen in their molecules, the second from 20 to 10, and the third below 10 per cent.

Thus table 7 clearly shows that the quantities of nitrogen nitrified are to a certain extent directly proportional to the percentage of nitrogen in the substance.

In the previous paper (1) while the relation between the nitrifiability and the nitrogen-carbon ratio was being considered it was remarked that the nitrogen-carbon ratio might be playing an important part in the nitrification of heterogeneous complex organic nitrogenous substances but that the nitrification of pure nitrogenous organic compounds appeared to be independent of the nitrogen-carbon ratio. By the pure nitrogenous organic compounds were meant the mono-amino-mono-carboxylic acids so far used and tryptophan. Among them the greater availability of a compound of a narrower ratio over that of a wider ratio was not so traceable. Further studies, however, with other pure organic compounds of nitrogen having more complex formulas than the simpler mono-amnio-mono-carboxylic acids showed that except for a few irregularities, the results indicate a fairly close relation between the nitrification values and the nitrogen-carbon ratios of the compounds as can be seen from table 8.

TABLE 8

Average results of nitrification and nitrogen-carbon ratio

NAME OF COMPOUNDS	AVERAGE PROPORTION OF NITROGEN NITRIFIED	NITROGEN CARBON RATIO
	per cent	
Nicotine	65.78	1:4.28
Arginine	62.16	1:1.28
Uric acid	61.52	1:1.07
Xanthine	58.17	1:1.07
Asparagine	57.58	1:1.71
Histidine dichloride	55.69	1:1.47
Aspartic acid	53.41	1:3.43
Lysine	52.77	1:2.57
Glutamic acid hydrochloride	50.45	1:4.28
Hippuric acid	41.42	1:7.71
Brucine	6.38	1:9.85
Strychnine	3.03	1:9.00

From table 8 it is clear that compounds having narrower nitrogen-carbon ratios are, in general, more readily nitrifiable than those of wider ratios. The opinion of Lipman, Brown, and Owen (5) that the substances with narrower nitrogen-carbon ratios are nitrified not only more rapidly but also more thoroughly is thus supported by these results.

From the average of the figures obtained for each compound a graded list of various substances used in these experiments can be prepared on the basis

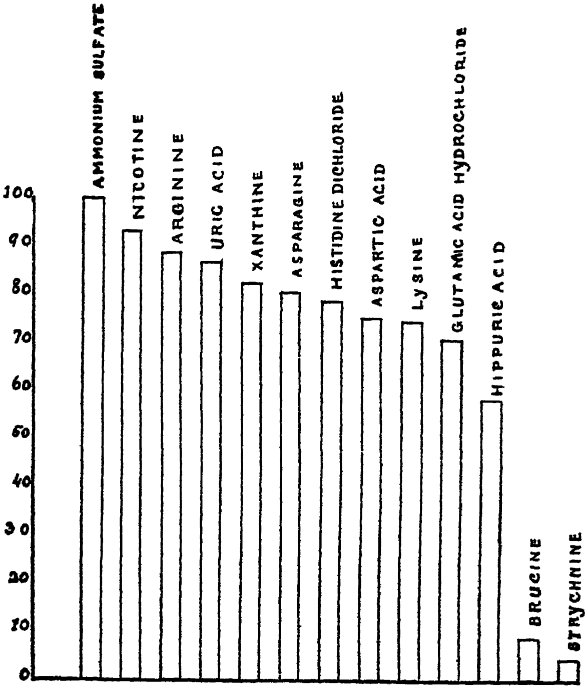


FIG. 1. RELATIVE NITROGEN AVAILABILITY OF SUBSTANCES USED IN EXPERIMENTS

TABLE 9

Fluctuations of the nitric nitrogen content in the soil of the control jars during different months of the year

DATE	NITRIC NITROGEN IN 100 GM. AIR-DRIED SOIL—30 DAYS INCUBATION PERIOD	DATE	NITRIC NITROGEN IN 100 GM. AIR-DRIED SOIL—40 DAYS INCUBATION PERIOD
	mgm.		*mgm.*
3/18/25	5.34	3/28/25	6.06
5/ 8/25	7.54	5/17/25	9.56
6/23/25	6.51	7/ 3/25	6.58
8/19/25	5.96	8/29/25	6.85
11/18/25	6.43	11/18/25	6.69
11/25/25	5.80	12/ 5/25	5.67
12/14/25	5.13	12/24/25	5.33
2/27/26	6.32	3/ 7/26	5.78

of their relative availability and is represented diagramatically in figure 1, the availability of ammonium sulfate being taken as 100.

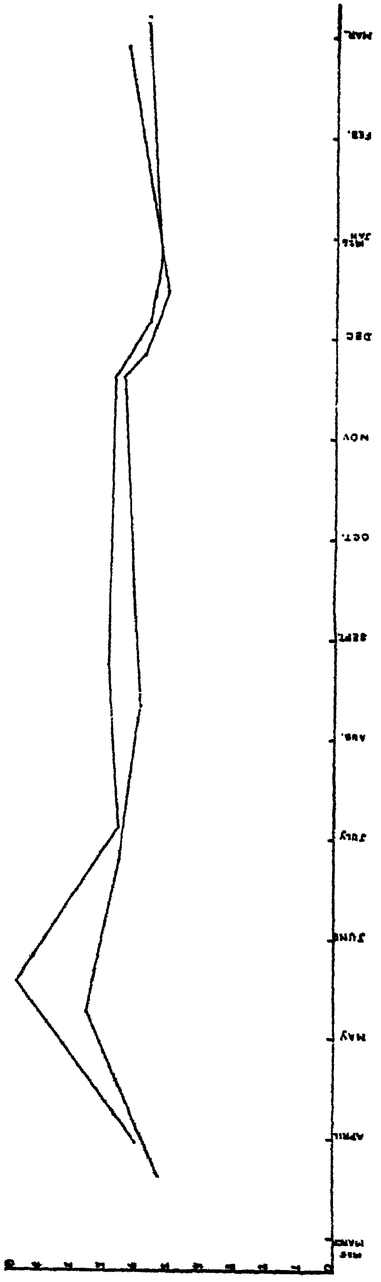


FIG. 2. NITRATE ACCUMULATION DURING DIFFERENT PARTS OF THE YEAR—MARCH, 1925, TO MARCH, 1926

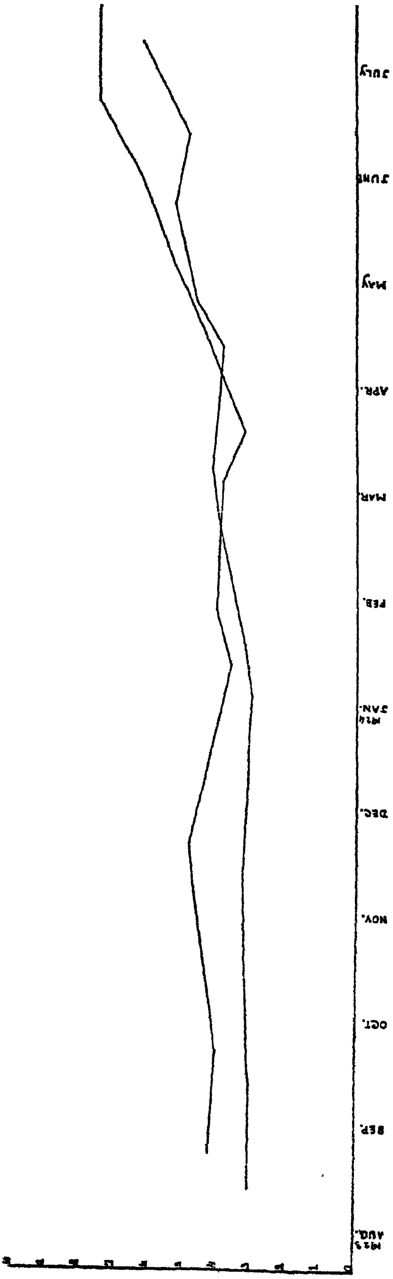


FIG. 3. NITRATE ACCUMULATION, CAWNPORE, AUGUST, 1923, TO JULY, 1924

TABLE 10

Results of hydrogen-ion determinations

pH VALUE DURING THIRTY DAYS									pH VALUE DURING FORTY DAYS								
Date	Nicotine	Brucine	Aspartic acid	Uric acid	Hippuric acid	Asparagine	Ammonium sulfate	Blank	Date	Nicotine	Brucine	Aspartic acid	Uric acid	Hippuric acid	Asparagine	Ammonium sulfate	Blank
3/19/25	7.1	6.8	6.6	.....	6.5	7.0	6.5	6.6	3/29/25	7.0	6.8	6.9	....	6.9	6.8	6.8	6.8
5/ 8/25	7.3	7.3	7.6	.....	7.4	7.2	7.4	7.4	5/18/25	7.5	7.5	7.6	....	7.6	7.5	7.5	7.6
6/24/25	7.6	7.5	7.2	7.4	7.6	.....	7.5	....	7/ 4/25	7.4	7.5	7.3	7.7	7.6	.....	7.5	7.3
8/20/25	7.3	7.6	7.4	7.3	7.4	.....	7.6	7.6	8/29/25	7.5	7.4	7.1	7.1	7.2	....	7.1	7.1
Average...	7.32	7.30	7.22	7.35	7.22	7.10	7.25	7.20		7.35	7.30	7.22	7.40	7.32	7.15	7.22	7.20
Date	Histidine dichloride	Arginine	Strychnine	Glutamic acid hydro-chloride	Xanthine	Lysine	Ammonium sulfate	Blank	Date	Histidine dichloride	Arginine	Strychnine	Glutamic acid hydro-chloride	Xanthine	Lysine	Ammonium sulfate	Blank
11/ 8/25	8.0	7.6	7.7	7.7	7.4	7.7	7.6	7.6	11/18/25	8.0	7.6	7.7	7.7	7.4	7.7	7.6	7.6
11/25/25	7.0	7.1	6.9	6.8	7.0	6.9	7.0	7.1	12/ 5/25	7.0	7.1	6.9	6.8	7.0	6.9	7.0	7.1
12/14/25	7.1	7.2	7.3	6.9	6.8	6.2	7.2	6.7	12/24/25	7.1	7.2	7.3	6.9	6.8	6.8	7.2	6.7
2/27/25	7.1	7.0	7.2	7.2	7.2	7.2	7.2	7.0	3/ 7/26	7.1	7.0	7.2	7.2	7.2	7.2	7.2	7.0
Average...	7.30	7.40	7.22	7.15	7.10	7.15	7.22	7.10		7.30	7.40	7.22	7.15	7.10	7.15	7.22	7.10

In the preceding paper (1) an attempt was made to study the seasonal variations of nitrates in the soil during the twelve months of the year. In the present investigations, although no separate experiments were carried out for the purpose, much information can be gained on the point from the nitrate contents of the control jars of the experiments. The results, given in table 9, are very similar to and therefore confirm the previous observations, which can be seen from the curves given in figures 2 and 3.

From the amounts of nitric nitrogen found in the control jars it can also be observed that the bacterial activity has not in any way decreased in the soil even when kept in storage for more than two years.

The hydrogen-ion determinations were made by the colorimetric method (2) using Sorenson's standards. The results obtained are given in table 10, from which it becomes apparent that there has been very little change in the pH values of the soil when treated with different kinds of organic compounds containing about 15 mgm. of nitrogen and incubated for either 30 or 40 days. The average figures of 30 days' incubation are almost identical with those of 40 days. The average of the various samples also agree very closely.

There is on the other hand a very little difference between the pH values of the control jars and those of others to which organic compounds were added. The latter have in general shown slightly higher pH values than the former; even the jars containing strychnine, where there was no nitrification, showed higher pH values.

SUMMARY

From these experiments of nitrification with pure nitrogenous organic compounds the following conclusions may be drawn:

1. A small portion of nitrogen from the ring compounds escapes determination after 3 hours of digestion with strong sulfuric acid. Prolonged digestion up to 24 hours converted nearly all the ring nitrogen into ammonium salts.
2. The members of the mono-amino-dicarboxylic acid series are slightly more nitrifiable than those of the mono-amino-mono-carboxylic acid series.
3. There is greater accumulation of nitric nitrogen from arginine than from lysine.
4. The data indicate the superiority of diamino-mono-carboxylic acids to mono-amino-dicarboxylic and mono-carboxylic acids as regards nitrifiability in the soil. They have also given higher results than those given by heterocyclic amino acids, which are only slightly better than the dicarboxylic acids.
5. Asparagine has shown better nitrifiability than aspartic acid.
6. The general trend of the nitrification of hippuric acid appears very similar to that of mono-amino-mono-carboxylic acids.
7. Brucine and strychnine have not only shown least nitrification values but have also given negative results. But the toxic effect of these poisonous compounds in the soil is only temporary and when they are removed the phenomenon of nitrification again takes places.
8. Xanthine and uric acid are proved to be the most readily nitrifiable organic compounds so far used except nicotine and arginine.
9. None of the nitrogenous organic compounds could compete with ammonium sulfate in nitrifiability.

10. It appears that the ring nitrogen is not only slowly and difficultly acted upon by strong acids used in the determination of total nitrogen by the Kjeldahl method, but also resists the action of the microörganisms of the soil to a greater extent than the chain nitrogen.

11. The nitrification of pure nitrogenous organic compounds is in some measure correlated to their composition and properties.

12. The compounds containing a larger percentage of nitrogen in their molecules produce larger amounts of nitrates, whereas compounds having a smaller percentage of nitrogen give rise to relatively smaller amounts of nitrates.

13. Organic compounds of nitrogen having more complex formulas than the simpler mono-amino-mono-carboxylic acids indicate a fairly close relationship between the nitrification values and the nitrogen-carbon ratios.

14. The bacterial activity has not in any way decreased in the soil even when kept in storage for more than two years.

15. The soil containing additional nitrogenous organic compounds shows slightly higher pH values than the soil without them.

REFERENCES

(1) Batham, H. N. 1925 Nitrification in soils. *Soil Sci.* 20: 337–351.
(2) Clark, W. M. 1915 The Determination of Hydrogen Ions. Baltimore, Md.
(3) Davis, W. A., and Sadtler, S. S. Allen's Commercial Organic Analysis, 7: 223–262.
(4) Dumer, R. B. 1917–20 Report on nitrogen determination. *Jour. Assoc. Off. Agr. Chem.* 3: 300.
(5) Lipman, J. G., Brown, P. E., and Owen, I. L. 1910 Relative availability of nitrogenous materials as measured by nitrification. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1910: 152–155.
(6) Schreiner, O., Reid, H. S., and Skinner, J. J. 1907 Certain organic constituents of soils in relation to soil fertility. U. S. Dept. Agr. Bur. Soils Bul. 47.
(7) Schreiner, O., and Shorey, E. O. 1910 Chemical nature of soil organic matter. U. S. Dept. Agr. Bur. Soils Bul. 74.
(8) Shorey E. C. 1913 Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. 88.
(9) Waksman, S. A. 1924 The influence of microörganisms upon the carbon-nitrogen ratio in the soil. *Jour. Agr. Sci.* 14: 560.

THE RELATION OF SOIL REACTION TO ACTIVE ALUMINUM[1]

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For several years there has been widespread interest in the aluminum content of the soil as it relates to soil acidity and crop injury. The presence in the soil of soluble aluminum compounds has generally been attributed to an acid condition of the soil. This has led to much discussion as to the origin of soil acids.

It is not the purpose of this paper to discuss this phase of the problem, but rather to consider the relationship which exists between acid soils and the presence of so-called active aluminum (3).

As excellent reviews and bibliographies have recently been published (4, 5, 7, 8), a full list of references need not be given here. In an earlier paper (1) it was pointed out that soil from an unlimed acid plot to which ammonium sulfate had been added annually for a period of years contained much more water-soluble aluminum than was found in a soil on a corresponding limed plot. It was further pointed out that crops on this land were a failure although commercial fertilizers were used in liberal amounts. This work led the authors to suspect that with the addition of increasing amounts of ammonium sulfate to a soil that was about neutral or slightly acid, there should be found, after a lapse of some time, increasing amounts of active aluminum in the soil. Conversely it was reasoned that with the addition of increasing amounts of basic material like ground limestone, to a strongly acid soil, there might be a gradual decrease in the amount of active aluminum.

In order that this question might be put to a test, two series of pot experiments were planned. The first provided for a normal soil to which increasing amounts of acid substances or acid-forming substances should be added, and the second provided for a strongly acid soil to which increasing amounts of basic materials or acid phosphate should be added.

SERIES 1. ACID MATERIALS

Soil 1 was a Sassafras loam having a pH value of 6.28 and an active aluminum content of 106 p.p.m. On December 8, 1925, 3½ pounds of this soil was placed in each of a series of pots with special treatment as indicated in

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[1] That soluble in 0.5 *N* acetic acid (3).

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table 1. The soil was kept under greenhouse conditions and water was added from time to time so that it was kept at about the optimum moisture condition. To one series of pots ammonium sulfate was added, to another inoculated sulfur and to another sulfuric acid. Determinations of pH values and active aluminum were made on samples taken from these pots on January 8, February 8, and April 8, 1926.

Ammonium sulfate. An examination of the table shows that up to April 8 the ammonium sulfate, with slight exception, had not greatly changed the pH value of the soil. On the other hand determinations made January 8 show a gradual increase in active aluminum from the check pot to the one that received 40 gm. of ammonium sulfate. The same is true of the results secured on the samples for February 8, though the values are for the most part smaller than those for January 8. The results for the final determination—April 8—four months from the beginning of the experiment, are in rather close agreement with those for February 8. Thus it would appear that the greatest changes took place during the first month.

TABLE 1

Influence of soil reaction on pH values and active aluminum—I. Normal loam soil, pH 6.28

POT NUMBER	SPECIAL TREATMENT	SAMPLED JANUARY 8		SAMPLED FEBRUARY 8		SAMPLED APRIL 8	
		pH	Active aluminum—Al_2O_3	pH	Active aluminum—Al_2O_3	pH	Active aluminum—Al_2O_3
			p.p.m.		*p.p.m.*		*p.p.m.*
1	Nothing	6.06	106	6.00	111.0	5.92	116
2	2.5 gm. $(NH_4)_2SO_4$	5.89	193	5.53	142.3	5.30	140
3	5.0 gm. $(NH_4)_2SO_4$	5.94	208	5.69	159.0	5.47	167
4	10.0 gm. $(NH_4)_2SO_4$	5.95	232	5.79	196.0	5.74	194
5	20.0 gm. $(NH_4)_2SO_4$	5.93	281	5.90	246.0	5.93	239
6	40.0 gm. $(NH_4)_2SO_4$	5.93	286	5.79	252.0	5.94	304
7	Nothing	6.06	106	5.84	105.0	5.94	109
8	1 gm. inoculated S	5.49	131	5.06	147.0	4.64	184
9	2 gm. inoculated S	5.24	134	4.09	229.0	3.98	347
10	4 gm. inoculated S	4.52	135	3.49	403.0	3.51	967
11	8 gm. inoculated S	4.29	134	3.23	928.0	3.15	1,278
12	16 gm. inoculated S	4.20	201	3.00	776.0	2.62	3,606
13	Nothing	6.06	106	5.71	104.0	5.83	112
14	5 cc. 1–5 H_2SO_4	4.87	147	4.60	275.0	4.92	209
15	10 cc. 1–5 H_2SO_4	4.21	429	4.15	386.0	4.26	.....
16	20 cc. 1–5 H_2SO_4	3.43	717	3.76	1,028.0	3.72	850
17	40 cc. 1–5 H_2SO_4	3.14	1,803	3.21	2,401.0	3.35	2,159
18	80 cc. 1–5 H_2SO_4	2.47	4,614	2.58	4,660.0	2.84	4,245

Inoculated sulfur. By referring to the determinations made January 8, it will be noted that as the amount of sulfur was increased the pH values decreased from 6.06 for the check to 4.2 with the highest amount of sulfur. Determinations made on the samples for February 8 and April 8 show still further decreases in pH values, the lowest pH, 2.62, being noted with the sample for April 8 where the application of sulfur was largest.

At the end of the first month not enough of the sulfur had been oxidized to influence greatly the amount of active aluminum, except in the case of the largest application. The figures for February 8 show a decided increase in the amount of active aluminum for the larger applications of sulfur. Two months later—April 8—still larger amounts of active aluminum were found where the sulfur was used, the highest amount being over 3600 p.p.m. with the largest application of sulfur. The increases, however, are not always directly proportional to the amount of sulfur used.

Sulfuric acid. By the end of the first month the sulfuric acid treatment had materially lowered the pH values, the largest amount used resulting in a pH of 2.47 as against 6.06 for the check. Likewise, determinations made on February 8 and April 8 show a gradual decrease in pH values with increase in amount of sulfuric acid, but with slight exception these figures are not essentially different from the figures of January 8. In this case also there is a very great increase in the amount of active aluminum with larger applications of sulfuric acid. In most cases the figures for the samples of February 8 are somewhat higher than those for the samples of January 8, but the increases from February 8 to April 8 are not especially significant. It would thus appear that with the sulfuric acid the most important changes took place within one month.

The criticism has been made that determinations of active aluminum mean very little, since such aluminum is not necessarily water-soluble. It would seem, however, that such determinations do have a value in that they indicate the soils that have aluminum in a form that may easily become water-soluble, or at least may become toxic to plants. The results reported indicate that an increase in acidity is followed by an increase in the amount of active aluminum, and with increase of active aluminum there is at least danger of toxicity. Whether such toxic compounds are in the form of soluble salts, or whether they exist as colloids is another question.

SERIES 2. INFLUENCE OF BASIC MATERIALS AND ACID PHOSPHATE

For the purpose of determining the effect of basic materials on active aluminum, a silt loam was chosen that was strongly acid and was known to contain more than the usual amount of active aluminum (pH 4.07, active-aluminum 781 p.p.m.). Three pounds of this soil was placed in each of a series of pots as in the previous experiment and was treated with increasing amounts of limestone, basic slag, and acid phosphate as indicated in table 2. The experiment was set up December 8, 1925, and samples were collected for the determination of pH values and active aluminum on January 8, February 8, and April 8, 1926.

Limestone. With the limestone treatment the pH values gradually rose until with 80 gm. of limestone to the pot it had reached 7.78 in one month. At the end of the fourth month the pH was 7.83.

With slight exception the active aluminum decreased gradually as the amount of limestone was increased. At the end of the first and second months the amount of active aluminum found was somewhat less with 40 than with 80 gm. of limestone. At the end of the fourth month the check pot showed 925 p.p.m. of active aluminum and the pot with 80 gm. of limestone, 283 p.p.m.

TABLE 2

The influence of reaction on pH values and active aluminum—II. Loam soil highly acid, pH 4.07

POT NUMBER	SPECIAL TREATMENT	SAMPLED JANUARY 8		SAMPLED FEBRUARY 8		SAMPLED APRIL 8	
		pH	Active aluminum—Al_2O_3	pH	Active aluminum—Al_2O_3	pH	Active aluminum—Al_2O_3
			p.p.m.		*p.p.m.*		*p.p.m.*
19	Nothing	4.16	795	4.09	799	4.02	925
20	5 gm. limestone	5.77	569	5.72	593	5.69	657
21	10 gm. limestone	6.77	469	6.64	445	6.66	433
22	20 gm. limestone	7.45	380	7.39	693	7.44	338
23	40 gm. limestone	7.70	371	7.49	305	7.69	307
24	80 gm. limestone	7.78	419	7.64	335	7.83	283
25	Nothing	4.16	795	4.03	861	4.15	932
26	5 gm. basic slag	5.41	445	5.36	480	5.42	420
27	10 gm. basic slag	5.94	297	5.97	295	6.05	239
28	20 gm. basic slag	6.84	194	6.65	177	6.89	132
29	40 gm. basic slag	7.58	142	7.45	89	7.52	82
30	80 gm. basic slag	8.21	169	7.95	216	8.05	70
31	Nothing	4.16	795	3.98	832	4.11	839
32	5 gm. acid phosphate	4.26	788	4.20	829	4.27	737
33	10 gm. acid phosphate	4.26	638	4.26	729	4.37	718
34	20 gm. acid phosphate	4.30	689	4.29	727	4.45	569
35	40 gm. acid phosphate	4.40	655	4.44	701	4.50	633
36	80 gm. acid phosphate	4.57	447	4.51	491	4.75	398

Basic slag. By using the same amounts of basic slage as of limestone the pH values were raised to a higher point than with the limestone, the maximum figures being 8.21 at the end of the first month, 7.95 at the end of the second month and 8.05 at the end of the fourth month. It is thus to be noted that there is little change in the pH values after the first month.

Concurrently with the changes in the pH values with basic slag there was a decrease in active aluminum. From about 800 p.p.m. in the beginning it was reduced to less than 100 p.p.m. at the end of four months. The samples for January 8 and February 8 show a higher content of active aluminum with 80 gm. of basic slag than with 40 gm. This same effect is noted all the way through with the limestone. It is probable that at the higher pH the aluminum again begins to go into solution.

Acid phosphate. As the amount of acid phosphate was increased the soil was slightly reduced in acidity. On January 8 the soil from the check pot showed a pH of 4.16 and that from the pot that received 80 gm. of acid phosphate, a pH of 4.57. The corresponding figures for the samples of February 8 were 3.98 and 4.51 and for April 8 they were 4.11 and 4.75. This finding proves the falsity of the belief that the continued use of acid phosphate tends to make the soil acid.

As the amount of acid phosphate used was increased the amount of active aluminum gradually decreased until with 80 gm. of acid phosphate to the pot it was brought to 447 p.p.m. on January 8, to 491 p.p.m. on February 8, and to 398 p.p.m. on April 8. The soil in the check pot for this series showed close to 800 p.p.m. active aluminum.

After the completion of the analytical work, germination tests were made with barley in all the pots. Briefly it may be stated that for the ammonium sulfate, sulfur, and sulfuric acid pots, the germination was good to poor in the first three pots of each series, and a failure in the fourth, fifth, and sixth pot of each series. In those pots that received the basic materials and acid phosphate, germination was fair to good in most cases. In a few cases it was reported very poor.

It is probable that no definite statement can be made as to the amount of active aluminum that will prove toxic to plants. For example, a soil that is noticeably acid and contains considerable active aluminum may be brought to the neutral point very soon by the addition of lime, but it may be some time before equilibrium between the bases in the soil is established and the amount of active aluminum reduced to a low figure. Active aluminum in relatively large amounts under such temporary conditions would not necessarily function as a toxic agent, since the lime would precipitate the water-soluble portion as rapidly as it was formed.

SERIES 3. NORMAL LOAM SOIL

In another experiment several compounds of ammonia (used in equivalent amounts of NH_3) were added to 3½ pounds of a Sassafras loam soil in each of a series of pots, two different amounts of compound being used in each case. The pots were allowed to stand in the greenhouse under optimum moisture and temperature conditions for about two months. Samples were then collected for pH and active aluminum determinations. The results of this work at the end of one month and again at the end of two months are shown in table 3. In the case of the sulfate, nitrate, and chloride the pH remained about constant or was lowered slightly. In the case of the remaining compounds the pH was increased very distinctly as compared with the check, and without exception, the double portion of the ammonium salts increased the pH as compared with the single portion.

In the majority of cases the large portion of ammonium salt gave more active aluminum than the small portion even though there was not a distinct increase in acidity. Out of seven cases there were three exceptions to this at the end of the first month and only one at the end of the second month. From this it would appear that increasing the amount of ammonium salt also increases the amount of active aluminum in the soil. The tendency for ammonium salts to increase the amount of active aluminum in soils may be explained on the theory of replaceable ions. The ammonium ions no doubt replace hydrogen ions from the zeolitic portions of the soil complex, and these in turn may act on aluminum and iron compounds in the soil.

TABLE 3

Influence of different ammonium compounds on soil reaction and active aluminum

POT NUMBER	SPECIAL TREATMENT	SAMPLED MARCH 2		SAMPLED MARCH 31	
		pH	Active aluminum—Al_2O_3	pH	Active aluminum—Al_2O_3
			p.p.m.		*p.p.m.*
1	Check	6.01	115	5.94	105
2	5 gm. ammonium sulfate, C.P.	5.93	161	5.82	138
3	10 gm. ammonium sulfate, C.P.	5.99	185	5.82	186
4	6.05 gm. ammonium nitrate, C.P.	5.92	128	6.10	116
5	12.10 gm. ammonium nitrate, C.P.	5.89	131	6.04	121
6	4.04 gm. ammonium chloride	5.97	126	6.01	114
7	8.09 gm. ammonium chloride	5.82	135	5.87	147
8	3.33 gm. ammonium phosphate $(NH_4)_2HPO_4$	6.77	97	6.37	95
9	6.66 gm. ammonium phosphate $(NH_4)_2HPO_4$	7.48	85	7.24	88
10	4.32 gm. ammonium carbonate	8.09	85	6.53	94
11	8.64 gm. ammonium carbonate	8.82	84	8.63	104
12	5.38 gm. ammonium oxalate	8.19	111	6.64	109
13	10.75 gm. ammonium oxalate	8.71	158	8.57	121
14	5.83 gm. ammonium acetate	8.47	113	6.93	86
15	11.66 gm. ammonium acetate	8.30	97	8.76	99
16	Check	6.38	106	6.01	85

SERIES 4. CYLINDER SOILS

In connection with the foregoing work it seemed worthwhile to determine the pH values and the active aluminum in some cylinder soils which have been under definite fertilizer treatment for nearly 30 years (2) and some field soils which have been under treatment for nearly 20 years (6). The cylinder soil is a Penn loam that has been receiving a definite fertilizer treatment annually since 1898. In this work one cylinder in each fertilizer series has been regularly limed at intervals of five years whereas the other cylinder of the series has been kept without lime. Cylinder 8 has received a complete fertilizer annually, the nitrogen being in the form of nitrate of soda; cylinder 17, a complete fertilizer, with nitrogen in the form of ammonium sulfate; and cylinder 19, a complete fertilizer, with nitrogen in the form of ammonium sulfate and with manure in addition. The results of the pH and active aluminum determinations made in 1925 are shown in table 4.

TABLE 4

Active aluminum and pH values for cylinder soils

CYLINDER NUMBER	SPECIAL TREATMENT	UNLIMED		LIMED	
		pH	Al_2O_3	pH	Al_2O_3
			p.p.m.		*p.p.m.*
8	Minerals + nitrate of soda	5.54	385	7.24	167
17	Minerals + ammonium sulfate	4.42	439	6.59	296
19	Minerals + manure + ammonium sulfate	4.81	439	6.33	325

TABLE 5

Active aluminum and pH values in soil from field plots

PLOT NUMBER	SPECIAL TREATMENT	UNLIMED		LIMED	
		pH	Al_2O_3	pH	Al_2O_3
			p.p.m.		*p.p.m.*
2	Muriate of potash	4.94	366	6.70	123
7	No fertilizer	4.79	345	6.96	108
9	Minerals + nitrate of soda	5.10	254	7.37	128
11	Minerals + ammonium sulfate	4.36	302	6.69	142
15	Minerals + tankage	4.75	303	7.23	113
18	Minerals + manure + nitrate of soda	5.30	222	7.25	119
19	Minerals only	4.97	303	7.15	145

It is noteworthy that soils from the unlimed cylinders show a low pH with fairly high active aluminum, whereas those from the limed cylinders show a comparatively high pH and low active aluminum. It may be pointed out that the soil which has received the ammonium sulfate, both limed and unlimed cylinders, shows a lower pH and higher active aluminum than the soil that has received nitrate of soda.

SERIES 5. FIELD SOILS

The field soils are of the Sassafras loam type and have been under observation and treatment since 1908. One section of plots has been limed at intervals of five years and the other has been left without lime. With the exception of plots 2 and 7 all have received an application of acid phosphate and muriate of potash (minerals) each year. In addition to this, plots 9, 11, 15, and 18 have received a nitrogenous fertilizer as indicated in table 5, and plot 18 has received annual applications of manure at the rate of 16 tons to the acre in addition. The figures for the pH values and active aluminum are shown in table 5.

Here as in the cylinder soils the unlimed soils show low pH values and high active aluminum, whereas with the limed soils the reverse is true. It is of interest to note that the ammonium sulfate plots (both limed and unlimed) show the lowest pH value for the respective series. The pH values for the unlimed series average slightly less than 5.00 whereas those for the limed series average a little more than 7.00. The active aluminum for the unlimed series averages more than 300 p.p.m. whereas for the limed series it is close to 125 p.p.m.

SUMMARY

Enough work has been done to show that acids and acid-forming materials decrease the pH and increase the amount of active aluminum in the soil.

Generally speaking, as the amount of these materials is increased there is an increase in active aluminum and decrease in pH, though in the case of the ammonium sulfate the decrease in pH was only slight.

When basic materials and acid phosphate were applied there was a decrease in amount of active aluminum and an increase in pH. With increase in the amount of lime and basic slag the decrease in active aluminum was more rapid than when the amount of acid phosphate was increased.

The results obtained in cylinder and field experiments where sulfate of ammonia has been used for a period of years confirm, in general, the results obtained in these pot experiments, though in the field and cylinder experiments no attempt was made to use different amounts of ammonium sulfate.

There is indication that sulfate of ammonia, used in large amount, or in moderate amount over a period of years, tends to increase the amount of active aluminum in the soil. When used over a period of years, without lime, this salt materially increases the hydrogen-ion concentration. It is suggested that there is a connection between this increase in active aluminum and certain toxic conditions of the soil which are sometimes met with.

REFERENCES

(1) Blair, A. W., and Prince, A. L. 1923 Studies on the toxic properties of soils. *Soil. Sci.* 15: 109–129.

(2) Blair, A. W., and Prince, A. L. 1924 Some changes in cylinder soils by long-continued crop and fertilizer treatment. *Soil Sci.* 18: 31–52.

(3) Burgess, P. S., and Pember, F. R. 1923 Active aluminum as a factor detrimental to crop production in many acid soils. R. I. Agr. Exp. Sta. Bul. 194.

(4) Hardy, F. 1926 The rôle of aluminum in soil infertility and toxicity. *Jour. Agr. Sci.* 16: 616–630.
(5) Line, J. 1926 Aluminum and acid soils. *Jour. Agr. Sci.* 16: 335–364.
(6) Lipman, J. G., Blair, A. W., and Prince, A. L. 1925 Field experiments on the availability of nitrogenous fertilizer, 1918–1922. *Soil Sci.* 19: 57–75.
(7) McGeorge, W. F. 1925 The influence of aluminum, manganese, and iron salts upon the growth of sugar cane and their relation to the infertility of acid island soils. Hawaii. Sugar Planters' Assoc. Exp. Sta. Agr. and Chem. Ser., Bul. 49.
(8) Magistad, O. C. 1925 The aluminum content of the soil solution and its relation to soil reaction and plant growth. *Soil Sci.* 20: 181–213.

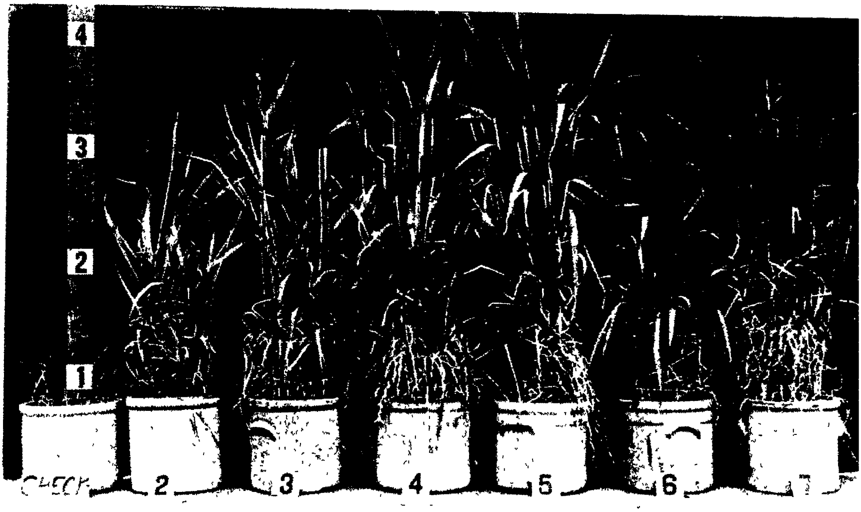
PLATE 1

BARLEY GROWN ON SOIL DESCRIBED UNDER SERIES 2 (pH 4.07; ACTIVE ALUMINUM 781 P.P.M.)

Check.
2. 12.5 gm. acid phosphate.
3. 25 gm. acid phosphate.
4. 125 gm. acid phosphate.
5. 250 gm. acid phosphate. (Equivalent to approximately 55,000 pounds acid phosphate to 2,000,000 pounds soil.)
6. 25 gm. finely ground limestone.
7. 25 gm. finely ground limestone and 125 gm. acid phosphate.

THE RELATION OF SOIL REACTION TO ACTIVE ALUMINUM
A. W. BLAIR AND A. L. PRINCE

PLATE



THE FERMENTATION CHARACTERS OF THE ROOT NODULE BACTERIA OF THE LEGUMINOSAE[1]

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Shortly after the isolation of the root nodule bacteria of leguminosae it was recognized that not all legumes are inoculated by the same culture of the organism. At first little if any difference in physiological and cultural characteristics was noted between any of the cultures from different plants. After more careful study, however, it became apparent that they were not exactly alike. Several workers have reported differences in size, morphology, flagellation, gum production, cultural characteristics, and serological properties between the cultures from different plants. By the use of these characteristic differences, experienced workers have been able to place the various strains in groups corresponding with the cross-inoculation groups.

Until recently it has been assumed that all the cultures inoculating any one legume are alike or at least very closely similar in regard to their morphological, cultural, and serological properties. Stevens (5) called attention to the fact that there are differences in cultural characteristics and serological properties between the cultures of any one cross-inoculation group. Wright (8) who worked with six strains of soybean bacteria[2] noted differences in the nitrogen-fixing power and was able to correlate these with the cultural and serological properties of the strains. Stevens was able to note similar differences in cultures of alfalfa bacteria, and to make similar correlations between nitrogen-fixing ability, cultural characteristics, and serological properties.

FERMENTATION OF SUGARS

Since the organisms show little ability to ferment sugars with the production of acid, few observations have been made on this character.

Zipfel (9) described the formation of acid by the nodule organism from both glucose and lactose. He found that the reaction of a legume-extract, sugar medium containing litmus, is sufficiently changed by the growth of the organism to cause it to become red. The type of organism used is not given.

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] For clarity of expression, the organisms will be referred to in this paper by the name of the legume species which they inoculate, i.e., the term "soybean bacteria" refers to the organism which produces nodules on soybeans.

Fred (3) found that small amounts of acid were formed in both nitrogenous and non-nitrogenous liquid media from sucrose, maltose, and mannitol. Four strains of alfalfa bacteria and two strains of clover bacteria were tested and no significant differences were noted. He found that from 0.2 cc. to 4.5 cc. of 0.1 *N* acid was produced in 100 cc. of culture media after one month's growth. The amount of acid was largely dependent on the medium used and was greater in the nitrogenous medium.

Fred and Davenport (4) in a later paper, 1918, reported that the hydrogen-ion concentration of a non-nitrogenous sucrose liquid medium may be increased as much as pH 0.4 by the growth of the root nodule bacteria. Twenty-five different cultures, including members of the alfalfa, clover, pea, bean, soybean, cowpea, and lupine groups, were tested.

Bialosuknia and Klott (2) and Bialosuknia (1) reported that there is a considerable variation in the degree of acidity formed by the different groups of the nodule bacteria and state that they believe it possible to classify these bacteria into groups by means of this character. No data are given to support these statements.[3]

Stevens (6) found that both hydrogen-ion concentration and titratable acidity were increased in a peptone-sucrose broth by the alfalfa bacteria. Eight strains were used and considerable differences were noted in their ability to produce acid.

Wright (8) in his study of the soybean bacteria, noted that these bacteria in sugar media of various kinds produce no increase in acidity. In many cases, there is a decrease in both hydrogen-ion concentration and total or titratable acidity. Litmus milk becomes strongly alkaline in reaction after 2 weeks incubation. Six strains of soybean bacteria were used and slight differences were noted in their behavior.

Stevens (7) suggested the use of litmus milk in the study of the legume nodule bacteria. Thirty cultures were tested on litmus, brom-cresol-purple, and Janus green milk. Organisms from the alfalfa, clover, pea, bean, cowpea, and soybean groups were included in these studies.

All the alfalfa cultures produced an acid reaction in litmus milk, whereas all of the other cultures either caused no change in reaction or produced an alkaline reaction. Differences were noted between the strains from any one cross-inoculation group, both with respect to the changing reaction and to the reduction of the dye. In milk, after 47 days incubation he found that one strain of alfalfa bacteria had changed the reaction from pH 6.55 to pH 5.18, and one strain of soybean bacteria had changed the reaction to pH 8.37. He states that acid production is much more rapid with freshly isolated strains. No indication is given as to whether this is a reflection of the rate of growth of the organism or whether it represents an actual change in its character.

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[3] In a personal communication from Dr. Bialosuknia he states that he has not been able to carry this work any further.

The literature dealing with the change in reaction which occurs in sugar media on the growth of the root nodule bacteria indicates that such changes, although small, occur with practically all cross-inoculation groups of the organism. Also it is indicated that there are differences in this character between the various cross-inoculation groups and also between the strains of each cross-inoculation group.

The following tests on the fermentation characters of the root nodule organism were carried out to determine the value of this character as an aid in the study of the organisms.

EXPERIMENTAL WORK

Sixty different cultures of the alfalfa, clover, pea, bean, cowpea, soybean, and dalea groups were used in these tests. All had been repeatedly tested for purity and for ability to produce nodules upon the corresponding legume. Included among these cultures are the ones which Stevens and Wright used in their studies on cultural characteristics, serological properties, and nitrogen-fixing ability.

Since *B. radiobacter* is frequently found as a contaminant and is extremely difficult to separate from the nodule bacteria, cultures have been included in the studies.

Three basic media, varying only with respect to nitrogen content, were used. One of these contained organic nitrogen, yeast infusion; one contained inorganic nitrogen, nitrate; and the third was nitrogen-free.

The composition of the basic nitrogen-free medium follows:

Di-basic potassium phosphate	0.5 gm.
Magnesium sulfate	0.2 gm.
Sodium chloride	0.2 gm.
Calcium carbonate	3.0 gm.
Agar	15.0 gm.
Organic carbon compound	10.0 gm.
Distilled water	1000 cc.
Brom thymol blue, 0.5 per cent alcoholic solution	5.0 cc.

The yeast medium contained 100 cc. per liter of yeast infusion[4] and the nitrate medium contained 0.5 gm. of potassium nitrate in one liter. Calcium carbonate was omitted from the media to which organic acids were to be added.

The ingredients for a medium, minus the organic carbon compound and indicator, were placed in a large flask and autoclaved for 15 minutes at 15 pounds pressure. The mixture was then filtered through a thick layer of absorbent cotton and the sugar or organic acid salt and indicator added. The reaction was adjusted to pH 7.2 and the media tubed. The media were sterilized in the autoclave at 15 pounds pressure for 15 minutes and the tubes slanted.

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[4] Fresh, starch-free bakers' yeast was made up to 10 per cent concentration with water and autoclaved until sterile. This was allowed to settle and the clear supernatant liquid used.

Inoculations were made with a platinum loop from fresh stock cultures.

The cultures were incubated at 28°C. for 2 weeks and then held at room temperature for at least 2 weeks. No attempt was made to make definite hydrogen-ion concentration readings on the cultures. Instead, the reaction of the medium was recorded as strongly acid, mediumly acid, slightly acid, very slightly acid, slightly alkaline, mediumly or strongly alkaline. In all cases where the reaction was recorded as strongly or mediumly acid, there was a clearing of the media due to the decomposition of the calcium carbonate.

FERMENTATION CHARACTERS OF THE ROOT NODULE BACTERIA IN NITRATE MEDIA

Melezitose, raffinose, trehalose, melibiose, maltose, lactose, sucrose, mannose, galactose, glucose, fructose, α-methyl-glucoside, rhamnose, arabinose, xylose, amydgalin, salicin, dextrin, sorbitol, dulcitol, mannitol, adonitol, erythritol, glycerol, sodium tartrate, sodium oxalate, sodium succinate, sodium malate, sodium lactate, sodium citrate, sodium acetate, sodium formate, and calcium pyruvate were used.

Two factors in the growth of the organisms may operate to change the hydrogen-ion reaction of the nitrate media used in these tests. Any fermentation of the carbohydrates with the production of an acid tends to increase the hydrogen-ion concentration. On the other hand the organisms in their growth absorb certain of the mineral constituents of the media. If the acidic radicals are absorbed in excess of the basic radicals, the hydrogen-ion concentration of the media tends to become lower and *vice versa*. Since the media used have a relatively low buffer capacity, the utilization of these radicals in small amounts may change the reaction considerably. In several cases, the media actually become more alkaline with the growth of the organism. The indications are that the organisms have not fermented the carbohydrate with the production of acid and that they have drawn more heavily on the acid than on the basic radicals. This condition is much more common in the media containing the nitrate than in either of the other media and it seems probable that it is the utilization of the nitrate radical by the organism which gives rise to this condition. A similar condition, however, is noted in the nitrogen-free media with some cultures. Here it is probably the use of the phosphate ion which accounts for the lowered hydrogen-ion concentration.

Tables 1 and 2 record the results of these tests with the various sugars.

The alfalfa bacteria as a general rule are more vigorous producers of acid than any of the other organisms. By the use of sucrose, xylose, mannose or galactose, the alfalfa bacteria may be separated from any of the other cross-inoculation groups. There is, however, within the group of alfalfa bacteria, considerable variation with respect to the production of acidity from the hexoses. Two general subgroups are evident. One of these, composed of 100,

TABLE 1

Fermentation characters of certain alfalfa, clover, bean, and dalea bacteria

2 weeks incubation—pentoses, hexoses, and allied carbohydrates in a nitrate medium

ORGANISM AND STRAIN NUMBER	PENTOSES			HEXOSES				DISACCHARIDES					TRISACCHARIDES		POLY-SAC-CHAR-IDES
	Rhamnose	Arabinose	Xylose	Fructose	Glucose	Galactose	Mannose	Sucrose	Lactose	Maltose	Melibiose	Trehalose	Raffinose	Melezitose	Dextrin
Alfalfa 100, 107, 109, 112, 115, 116, 117 and 118	+++	++++	+++	+	++++	++++	+++	+++	++	++	+++	++	++	++	−−−
Alfalfa 101, 102, 103, 104, 105	+++	++++	+++	−−−	++++	++++	+++	+++	−−−	+	−−	−−	−−−	−	−−−
Alfalfa 106, 108	+++	++++	+++	0	++++	++++	+++	++	++	−−	+++	++	++	++	−−−
Alfalfa 113, 114	+++	+++	+++	+	++++	++++	+++	++	++	++	−−	++	−	−	−−−
Alfalfa 110	+++	++++	+++	+	++++	++++	+++	+++	++	++	+++	++	−	−−	−−−
Alfalfa 111	+++	++++	+++	+	++++	++++	+++	+++	++	++	+++	++	++	−−	−−−
Clover 123, 124, 125	++	+++	0	+	+++	++	++	0	+	++	0	−	0	0	−−−
Clover 121, 122, 127	++	+++	0	+	+++	++	++	0	+	++	−	−	0	0	−−−
Clover 125A, 126	++	+++	0	−	+++	++	++	0	+	++	−	−	0	0	−−−
Bean 140	+	++++	+	−	+	+	+	+	+	0	0	0	−−	0	−−−
Bean 145	+	++++	+	+	+	+	+	+	+	0	0	0	0	0	−−−
Bean 146, 147, 148, 149	+	+	+	+	+	+	+	+	+	0	0	0	−−	0	−−−
Dalea 170, 171	++++	++	−−	−	+++	0	+	−	+	0	−−−	−−−	−	−−	−−−

++++, strongly acid; +++, mediumly acid; ++, slightly acid; +, very slightly acid; −−−, strongly alkaline; −−, mediumly alkaline; −, slightly alkaline; 0, no change in reaction.

Fermentation characters of certain pea, soybean, and cowpea bacteria and B. radiobacter

2 weeks incubation—pentoses, hexoses, and allied carbohydrates in a nitrate medium

ORGANISM AND STRAIN NUMBER	PENTOSES			HEXOSES				DISACCHARIDES					TRISACCHARIDES		POLYSACCHARIDES
	Rhamnose	Arabinose	Xylose	Fructose	Glucose	Galactose	Mannose	Sucrose	Lactose	Maltose	Melibiose	Trehalose	Raffinose	Melezitose	Dextrin
Pea 130	++	++++	0	+	+++	++	++	++	+	++	−	+	−−	0	−−−
Pea 131	++	++	0	+	+++	++	++	++	+	++	−	+	−−	0	−−−
Pea 132	++	++++	0	+	+++	++	++	++	+	++	0	0	0	0	−−−
Pea 133	++	++	0	+	+++	++	++	++	+	++	0	+	0	0	−−−
Pea 135	++	++	++	+	+++	++	++	0	+	++	−	+	−−	0	−−−
Pea 134	++	++++	0	+	+++	++	++	++	+	++	−	0	0	0	−−−
Pea 136	++	++	0	+	+++	++	++	++	+	++	0	+	0	0	−−−
Pea 5	++	++	++	+	+++	++	++	++	+	++	0	+	0	0	−−−
Pea 10	++	++++	0	+	+++	++	++	++	+	++	−	+	−−	0	−−−
Pea 15	++	++	0	+	+++	++	++	+	+	++	−	+	−−	0	−−−
Pea 20, 21, 22, 23	++	++++	0	+	+++	++	++	++	+	++	−	0	−−	0	−−−
Soybean 160, 164, 168	−−	+++	−	−−−	−−−	−−−	−−−	−−	−	−−−	−−	−	−−	0	−−−
Soybean 161, 162, 163, 165, 166	−−	0	−	−−−	−−−	−−−	−−−	−−	−	−−−	−−	−	−−	0	−−−
Cowpea 143	−−	+	−−	−−−	−−−	−−−	−−−	−−	−	−−−	−−	−	−−	0	−−−
Cowpea 170	−−	+++	−−	−−−	−−−	−−−	−−−	−−	−	−−−	−−	−	−−	0	−−−
Cowpea 142	−−	++++	−−	−−−	−−−	−−−	−−−	−−	−	−−−	−−	−	−−	0	−−−
B. radiobacter	++++	++++	++	+++	+++	++++	++++	−	+	0	−−−	−−−	−−	−−	++++

++++, strongly acid; +++, mediumly acid; ++, slightly acid; +, very slightly acid; −−−, strongly alkaline; −−, mediumly alkaline; −, slightly alkaline; 0, no change in reaction.

107, 109, 112, 115, 116, 117, and 118, is characterized by a vigorous production of acid. Another such group, composed of 101, 102, 103, 104, and 105, is characterized by its inability to produce acid from melezitose, raffinose, trehalose, melibiose, lactose, and fructose. The remaining six strains of the alfalfa group which were tested are intermediate between these two extremes.

The clover group of bacteria and the pea group of bacteria in a general way react very similarly on these carbohydrates. Considerable variation is shown among the individuals of each group, but no general grouping is possible as with the alfalfa bacteria. The clover and pea bacteria produce acid from most of the carbohydrates in this list, but in no case is there a vigorous fermentation. The clover and pea groups of bacteria may be separated from each other by their reaction on sucrose media.

The bean bacteria produce even smaller quantities of acid than either the clover or pea bacteria although slighly acid reactions are noted. The use of either maltose or glucose serves to separate the members of this group from any of the others.

Soybean and cowpea bacteria react similarly. No acid is produced from any carbon compounds[5] except arabinose, and in practically every case the medium becomes distinctly alkaline. However, with arabinose the reaction was first strongly alkaline and later became acid. The same condition prevails with xylose but the change from alkaline to acid is much slower. Xylose and arabinose permit a more vigorous growth of the soybean and cowpea bacteria than do any of the other compounds tested.

Dalea bacteria are very vigorous growers and in almost all cases the first reaction, after 3 to 5 days, is slightly alkaline, particularly on the upper slope of the slant. Later, acid is produced from some of the sugars to overcome the initial alkaline reaction and to leave an acid reaction.

B. radiobacter behaves much the same as dalea bacteria, but it is a more vigorous acid producer with several of the carbohydrates.

The hexoses are more easily fermented with the production of acid than are any of di- or tri-hexoses. Fructose is not utilized nearly so readily as the aldehyde hexoses. None of the legume bacteria are able to produce an acid reaction when grown on dextrin. The *B. radiobacter* strain used produces a very strong acid reaction on this substance. Three other strains of *B. radiobacter* were secured from Dr. Löhnis of the United States Department of Agriculture. Two were able to ferment dextrin, and one was unable to do so.

Tables 3 and 4 record the results of the tests with certain polyhydric alcohols, glucosides, and organic acid salts.

A similar relationship holds with the glucosides and alcohols as with the sugars. The alfalfa, clover, pea, and bean bacteria produce acid from most of these compounds. Of these the alfalfa bacteria are the strongest acid producers. Here again the same division of the alfalfa bacteria into two

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[5] Upon prolonged incubation, 6 weeks to 2 months, many of the cultures in these groups produce a light acid reaction.

TABLE 3

Fermentation characters of certain alfalfa, clover, bean, and dalea bacteria

2 weeks incubation—polyhydric alcohols, glucosides, and organic acid salts in a nitrate medium

ORGANISM AND STRAIN NUMBER	POLYHYDRIC ALCOHOLS						GLUCOSIDES			ORGANIC ACID SALTS					
	Glycerol	Erythritol	Adonitol	Mannitol	Dulcitol	Sorbitol	α-methyl glucoside	Amydalin	Salicin	Sodium tartrate	Sodium succinate	Sodium malate	Sodium lactate	Sodium citrate	Sodium formate
Alfalfa 100, 107, 109, 112, 115, 116, 117, 118	+++	+	0	+++	−−	+++	−−	+++	++	−−	−−−	−−−	−−−	0	−−−
Alfalfa 101, 102, 103, 104, 105	−−−	−−	0	−−	−−	−−−	−−	0	−−	−−	−−−	−−−	−−−	0	−−−
Alfalfa 106, 108	−−−	+	++	++	−−	+++	−−	0	++	−−	−−−	−−−	−−−	0	−−−
Alfalfa 113, 114	−−−	−−	−−−	++	−−	−−	−−	0	0	−−	−−−	−−−	−−−	0	−−−
Alfalfa 110	+++	+	0	+++	−−	+++	−−	+++	++	−−	−−−	−−−	−−−	0	−−−
Alfalfa 111	+++	+	++	+++	−−	+++	−−	+++	++	−−	−−−	−−−	−−−	0	−−−
Clover 123, 124, 125	++	+	0	++	+	++	−−	0	0	−−−	−−	0	−	0	−−−
Clover 121, 122, 127	−−	−	0	−	+	0	−−	0	0	0	−−−	−−−	−−−	−−	−−−
Clover 125A, 126	++	−	0	++	+	++	−−	0	0	−	−−	0	−−−	0	−−−
Bean 140	−	−	−	++	−	−	−−	0	0	0	−−−	−−−	−−−	0	−−−
Bean 145	++	−	+	+++	+	++	−−	0	0	0	0	0	0	0	−−−
Bean 146, 147, 148, 149	−	−	−	++	−	+	−−	0	0	−	−−−	0	−−	0	−−−
Dalea 170, 171	0	−	−	−−	−	−−	−−	0	0	−	−−−	−−−	−−−	−−	−−−

++++, strongly acid; +++, mediumly acid; ++, slightly acid; +, very slightly acid; −−−, strongly alkaline; −−, mediumly alkaline; −, slightly alkaline; 0, no change in reaction.

TABLE 4

Fermentation characters of certain pea, soybean, and cowpea bacteria and B. radiobacter

2 weeks incubation—polyhydric alcohols, glucosides, and organic acid salts in a nitrate medium

ORGANISM AND STRAIN NUMBER	POLYHYDRIC ALCOHOLS						GLUCOSIDES			ORGANIC ACID SALTS					
	Glycerol	Erythritol	Adonitol	Mannitol	Dulcitol	Sorbitol	α-methyl glucoside	Amydalin	Salacin	Sodium tartrate	Sodium succinate	Sodium malate	Sodium lactate	Sodium citrate	Sodium formate
Pea 130	−	+	−−	−	0	++	−−	0	0	−−−	−−−	0	−	0	−−−
Pea 131	++	−	0	++	+	++	−−	0	0	0	−−−	−−−	−−	−−	−−−
Pea 132	++	−	0	++	0	++	−−	0	0	−−−	−−−	0	−	0	−−−
Pea 133	++	+	0	++	+	++	−−	0	0	−−−	−−−	0	−	0	−−−
Pea 135	++	+	−−	++	0	++	−−	0	0	0	−−−	−−−	−−	0	−−−
Pea 134	++	+	−−	+++	+	++	−−	0	0	−−−	−	0	−	0	−−−
Pea 136	++	+	0	++	0	++	−−	0	0	0	−−−	0	−−	0	−−−
Pea 5	−	−	0	++	0	++	−−	0	0	−−−	−−−	0	−	0	−−−
Pea 10	−	−	−−	++	0	++	−−	0	0	−−−	−−−	0	−	0	−−−
Pea 15	−	−	0	−	0	++	−−	0	0	0	−−−	−−−	−−−	−−−	−−−
Pea 20, 21, 22, 23	++	−	0	++	0	++	−−	0	0	−−−	−	0	−	0	−−−
Soybean 160, 164, 168	−−−	−	−−−	−−	−−	−−	−−	−−	−−−	−−−	0	−−	−−−	0	−−−
Soybean 161, 162, 163, 165, 166	−−−	−	−−−	−−	−−	−−	−−	−−	−−−	−−−	0	−−	−−−	0	−−−
Cowpea 143	−−−	−	−−−	−−−	−−	−−	−−	−−	−−−	−−−	−−−	−−−	−−−	0	−−−
Cowpea 170	−−−	−	−−−	−−	−−	−−	−−	−−	−−−	−−−	−−−	−−−	−−−	0	−−−
Cowpea 142	−−−	−	−−−	−−	−−	−−	−−	−−	−−−	−−−	0	−−−	−−−	0	−−−
B. radiobacter	++++	++	++++	+++	++	−−	−−	+++	+++	+++	−−−	−−−	−−−	++	−−−

++++, strongly acid; +++, mediumly acid; ++, slightly acid; +, very slightly acid; −−−, strongly alkaline; −−, mediumly alkaline; −, slightly alkaline; 0, no change in reaction.

groups, was noted. The weakest acid producers are the bean bacteria, whereas the clover and pea bacteria are intermediate.

The soybean and cowpea bacteria in most cases produce a strongly alkaline reaction. Dalea bacteria in every case first produce a strongly alkaline reaction, which in practically every case is followed by an acid reaction.

With the glucosides and alcohols as with the sugars, considerable variation is noted among the fermentation characters of the members of each group. The polyhydric alcohols bring out these differences very clearly.

With the salts of the organic acids, the utilization of the acid radical usually results in a lowered hydrogen-ion concentration. In some cases with acids of high molecular weight it is possible that the acid might be split into two or more acids of lower molecular weight and thus even increase the hydrogen-ion concentration.

In addition to the organic acid salts listed in the tables, sodium oxalate, sodium acetate, and calcium pyruvate were tested. Neither the oxalate nor the acetate ions were utilized by any of the organisms tested. In the case of the pyruvate ion slight growth, with the production of a light alkaline reaction, was secured with the following strains: alfalfa 100, 102, 104, 107, 108, and 117, clover 125A; pea 132, 133, 136, and 5; and soybean 160, 162, and 163. The reaction of these organisms to the pyruvate ion is of interest. Normally this compound is thought to be broken down very readily by bacterial action. Only very few of the legume bacteria, however, were able to utilize it in these tests.

All of the other acids tested supported the growth of the organisms. In practically every case where a vigorous growth was secured the reaction of the medium became alkaline. However the final reaction of the medium cannot be taken as a measure of the growth of the organism. In some cases a marked lowering of the hydrogen-ion concentration occurred with a very scanty growth, whereas in others a vigorous growth was accompanied by little change in the reaction of the medium.

Sodium malate, sodium succinate, and sodium lactate supported better growth than did any of the other organic acids. None of the organic acids was as good a carbon source as were many of the carbohydrates and polyhydric alcohols.

Little difference in fermentation characters was noted among the strains of the alfalfa, soybean, and cowpea bacteria.

B. radiobacter showed the interesting phenomena of producing acid reactions on the media containing sodium tartrate and sodium citrate.

FERMENTATION CHARACTERS OF LEGUME BACTERIA WITH YEAST INFUSION MEDIA

Lactose, maltose, sucrose, glucose, galactose, fructose, xylose, arabinose, mannitol, glycerol, salicin, sodium malate, and sodium formate were tested in the yeast medium. Table 5 records the data on these tests.

TABLE 5

Fermentation characters of certain legume bacteria and B. radiobacter

2 weeks incubation—carbohydrates, polyhydric alcohols, and organic acid salts in a yeast infusion medium

ORGANISM AND STRAIN NUMBER	PENTOSES		HEXOSES			DISACCHARIDES			POLYHYDRIC ALCOHOLS		POLY-SACCHARIDE	ORGANIC ACID SALTS	
	Arabinose	Xylose	Fructose	Glucose	Galactose	Sucrose	Maltose	Lactose	Mannitol	Glycerol	Salicin	Sodium malate	Sodium formate
Alfalfa 100, 107, 109, 111, 112, 115, 116, 117, 118	++++	++++	+++	++++	++++	+++	+++	+++	++++	++	+++	— — —	— — —
Alfalfa 101, 102, 103, 104, 105	++++	++++	0	++++	++++	+++	+	++	0	++	0	— — —	— — —
Alfalfa 106	++++	++++	0	++++	++++	+++	+	++	++++	++	+++	— — —	— — —
Alfalfa 108	++++	++++	+++	++++	++++	+++	+	++	++	++	+++	— — —	— — —
Alfalfa 110	++++	++++	+++	++++	++++	+++	+	+++	++++	++	+++	— — —	— — —
Alfalfa 113, 114	++++	++++	0	++++	++++	+++	+	+++	++++	++	0	— — —	— — —
Clover, all strains	+	++	+	+	+	++	+	++	+++	++	0	— — —	— — —
Pea 135	+	++	+	++	+	++	+	++	+++	++	0	— — —	— — —
Pea 134	+	+	+	+	+	+	+	++	++	++	0	— — —	— — —
Pea, all other strains	++	++	+	++	+	++	+	++	+++	++	0	— — —	— — —
Bean, all strains	++	++	+	+	+	++	+	+	+++	++	0	— — —	— — —
Dalea 170, 171	+++	+	+	+	++	++	+	+	+++	++	0	— — —	— — —
Soybean, all strains	— — —	— — —	— — —	— — —	— — —	— —	— — —	— — —	— — —	— — —	— —	— — —	— — —
Cowpea, all strains	— — —	— — —	— — —	— — —	— — —	— —	— — —	— — —	— — —	— — —	— —	— — —	— — —
B. radiobacter	+++	++++	+++	+++	++++	++	+++	+	+++	++	0	— — —	— — —

++++, strongly acid; +++, mediumly acid; ++, slightly acid; +, very slightly acid; — — —, strongly alkaline; — —, mediumly alkaline; —, slightly alkaline; 0, no change in reaction.

Because of the higher buffer capacity of the yeast infusion as compared to the nitrate medium there was less change in the hydrogen-ion concentration. Also it removed the possibility of a lowering of the hydrgoen-ion concentration because of an excess absorption of nitrate ions by the culture. On the other hand, the yeast infusion media in many cases appeared to support a much better growth than the nitrate media.

These differences are reflected in the reaction of the cultures. In most cases the acid-producing cultures produce higher hydrogen-ion concentrations in the yeast infusion media than in the corresponding nitrate media. Differences between strains within the cross-inoculation groups are not nearly so pronounced as they were in the nitrate media. The two subgroups within the alfalfa bacteria can still be detected, although the differences are not so decisive. No differences are apparent within the clover group. Two of the pea strains react slightly differently from the others. The dalea bacteria show perhaps the greatest change from their reaction on the nitrate media. In the yeast infusion media the cultures produce considerable amounts of acid without first passing through the alkaline stage.

TABLE 6

Fermentation characters of certain legume bacteria and B. radiobacter

2 weeks incubation—hexoses, pentoses, and mannitol in a nitrogen-free medium

ORGANISM AND STRAIN NUMBER	LACTOSE	SUCROSE	GLUCOSE	XYLOSE	ARABINOSE	MANNITOL
Alfalfa 101, 102, 103, 104, 105	+	0	++	++	++	0
Alfalfa, all other strains	+	0	++	++	++	+
Clover, all strains	0	0	+	+	+	0
Pea, all strains	0	0	+	+	+	0
Bean, all strains	0	0	+	+	+	0
Soybean, all strains	−	−	−−	−−	−−	−
Cowpea, all strains	−	−	−−	−−	−−	−
Dalea, all strains	+	0	++	+	+	0
B. radiobacter	+	+	++	+	+	0

++++, strongly acid; +++, mediumly acid; ++, slightly acid; +, very slightly acid; −−−, strongly alkaline; −−, mediumly alkaline; −, slightly alkaline; 0, no change in reaction.

FERMENTATION CHARACTERS OF LEGUME BACTERIA IN NITROGEN-FREE MEDIA

Lactose, sucrose, glucose, xylose, arabinose, and mannitol were tested in a nitrogen-free basic medium. The results of these tests are recorded in table 6.

The growth in this medium was not so vigorous as in the corresponding media in the other series, nor was the change in hydrogen-ion concentration so marked. The alfalfa bacteria group is the only one that shows any division into subgroups. On mannitol there is a clear-cut separation into two subgroups. In each of the other groups all of the strains react essentially alike.

The alfalfa and dalea bacteria are the most vigorous acid producers. Clover, pea, and bean bacteria produce acid from glucose, xylose, and arabinose. The soybean and cowpea bacteria produce an alkaline reaction in all of the media tested.

SUMMARY

The fermentation characters of 60 cultures of root nodule bacteria, including members of the alfalfa, clover, pea, bean, soybean, cowpea, and dalea groups, and *B. radiobacter* have been determined. Agar media of low buffer capacity containing brom-thymol-blue as a hydrogen-ion indicator were used.

The fermentation characters of these organisms are sufficiently definite and pronounced to enable the separation into groups corresponding to the cross-inoculation groups. An exception to this statement is found in the failure to separate the soybean and cowpea groups.

By means of their fermentation characters the root nodule bacteria may be divided into two broad groups; namely, the acid producers, which include the alfalfa, clover, pea, bean, and dalea bacteria; and those which lower the hydrogen-ion concentration of the culture media, including the soybean and cowpea bacteria. This separation corresponds to that made on the basis of flagellation. The members of the first group are peritrichous whereas those of the second group have polar flagella.

In many of the cross-inoculation groups, subdivisions have been established. The subdivisions which are established within the alfalfa and clover groups by their fermentation characters are definite and clear cut. These groups correspond with the groups which have been established by other workers by means of cultural and serological tests. Considerable variation was exhibited among the members of each of the other cross-inoculation groups in their fermentation characters. In these groups, however, the differences between strains were less constant and gave less opportunity for classification into subgroups.

B. radiobacter may be distinguished from any of the root nodule bacteria by its strong acid fermentation of dextrin.

Comparison of the reactions secured on nitrate, yeast infusion, and nitrogen-free media shows that these organisms produce larger amounts of acid on nitrogenous media. This is probably merely a reflection of a more vigorous growth.

Many of the cultures apparently withdraw acid radicals from the medium much more rapidly than they do basic radicals. A comparison of the reactions secured on the nitrate and non-nitrogenous media indicates that the nitrate radical, in particular, is rapidly withdrawn from the culture media by the organisms. The fact that many of the cultures produce an alkaline reaction in non-nitrogenous media indicates that some other acid radical than nitrate is also heavily drawn upon. Evidence from other investigations would indicate that the phosphate radical is used in considerable amounts.

REFERENCES

(1) Bialosuknia, W. 1923 Investigations on *Bacterium radicicola*. *Rocz. Nauk Rolnicz.* 10: 142–147.

(2) Bialosuknia, W., and Klott, C. 1923 Investigations on *Bacterium radicicola*. *Rocz. Nauk Rolnicz.* 9: 288–335.

(3) Fred, E. B. 1911 A physiological study of the legume bacteria. *Ann. Rpt. Va. Agr. Exp. Sta.* 1911: 145–173.

(4) Fred, E. B. and Davenport, A. 1918 Influence of reaction on nitrogen-assimilating bacteria. *Jour. Agr. Res.* 14: 317–336.

(5) Stevens, J. W. 1923 Can all strains of the specific organism be recognized by agglutination? *Jour. Infect. Diseases* 33: 557–566.

(6) Stevens, J. W. 1925 A study of various strains of *Bacillus radicicola* from nodules of alfalfa and sweet clover. *Soil Sci.* 20: 45–65.

(7) Stevens, J. W. 1925 The value of litmus, brom-cresol purple and Janus-green milk in a study of the nodule organisms of leguminosae. *Jour. Agr. Res.* 31: 997–1000.

(8) Wright, Wm. H. 1925 The nodule bacteria of soybeans. *Soil Sci.* 20: 95–130; 131–141.

(9) Zipfel, H. 1911 Beiträge zur Morphologie und Biologie der Knöllchenbakterien der Leguminosen. *Centbl. Bakt.* (II) 32: 97–137.

THE EFFECT OF TEXTURE OF SANDY SOILS ON THE MOISTURE SUPPLY FOR CORN DURING SEASONS OF FAVORABLE AND UNFAVORABLE DISTRIBUTION OF RAINFALL[1]

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The productive capacity of soils in any region is dependent upon the amount of available moisture at the disposal of crops during the growing season. The supply of available moisture is largely determined by the amount and distribution of rainfall and by the textural quality of the soil in its effect on absorbing, retaining, and delivering moisture to crops. It is the object of this paper to show the effect of texture of sandy soils on the production of corn under conditions of favorable and unfavorable distribution of rainfall.

For each major class of soil there is an optimum moisture content at which the physical, biological, and chemical phenomena are most active, thereby usually providing conditions for maximum growth, and any marked departure from the optimum in either direction results in decreased plant growth.

The mean annual rainfall for Wisconsin, as cited by Whitson and Baker (3), is 31 inches, about half of which comes in May, June, July, and August, and nearly 70 per cent from April to September inclusive. In spite of this favorable mean distribution, periods frequently occur during which distribution is unfavorable for the maintenance of an optimum supply of moisture in the sandy soils of the state.

In a previous paper, the author (2) discussed the effect of texture of sandy soils on utilization of moisture in crop production as primarily related to the distribution of rainfall, and it was during this investigation that some observations on corn were made during periods of limited rainfall. Subsequent wilting coefficient determinations were made of these soils, the results of which, together with observations under out-of-door conditions, are given.

EXPERIMENTAL CONDITIONS

Inasmuch as the observations were made on the experiments I and II previously reported, the reader is referred to the author's previous paper (2, p. 199–204), for a description of the cylinders, soils, fertilizer treatments, and moisture determinations. The amount and distribution of rainfall during the growing seasons covered by these experiments are also given in that paper (2, p. 205, 206).

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Wilting coefficient determinations were conducted under greenhouse conditions in the late spring of 1924, with corn as the indicative crop. The galvanized iron containers used were 12 inches deep and 6 inches in diameter and their interior surfaces were covered with two coats of asphaltum paint. Each cylinder was supplied with a glass asperating tube, the lower end of which was embedded at the bottom of the container in a small ball of glass wool to avoid stoppage by soil.

TABLE 1

Mechanical composition of soils used in experiment IV, from field plots near Hancock

DEPTH	FINE GRAVEL	COURSE SAND	MEDIUM SAND	FINE SAND	VERY FINE SAND	SILT	CLAY	TOTAL
inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Coloma sand								
0–16	0.29	16.28	40.07	29.14	4.32	5.80	3.88	99.78
6–12	0.40	20.58	42.74	24.76	3.71	4.85	3.60	100.64
12–18	0.45	17.86	42.60	28.69	3.07	4.29	3.54	100.50
18–24	0.40	20.15	44.28	27.05	2.32	3.08	2.89	100.17
24–30	0.47	19.04	41.88	30.06	3.13	2.48	2.59	99.65
30–36	0.37	19.67	42.18	30.26	3.01	2.33	2.25	100.07
36–40	0.37	15.77	40.13	34.04	4.96	2.66	2.24	100.07
Coloma fine sand								
0–6	0.11	7.32	27.31	51.53	5.92	4.86	4.66	101.71
6–12	0.19	7.31	25.47	52.36	6.05	4.65	3.67	99.70
12–18	0.17	7.36	24.96	54.41	5.22	3.96	3.45	99.53
18–24	0.16	8.11	25.56	54.17	5.71	2.89	3.55	100.24
24–30	0.10	6.33	23.08	58.86	6.60	2.00	3.07	100.04
30–36	0.30	8.46	26.62	55.30	6.34	1.57	2.32	100.91
36–40	0.16	6.36	24.57	59.91	5.81	1.33	1.84	99.58
Coloma sandy loam								
0–6	0.30	9.92	20.82	36.41	13.69	14.08	4.85	100.07
6–12	0.27	8.79	20.61	40.52	13.48	11.71	4.37	99.75
12–18	0.49	11.77	23.77	38.37	12.38	9.17	4.10	100.05
18–24	0.30	12.65	24.70	36.71	11.62	7.90	6.26	100.14
24–30	0.31	12.18	22.57	35.20	13.67	9.02	7.76	100.71
30–36	0.64	14.49	26.12	31.90	10.78	8.64	7.61	100.18
36–40	0.68	13.51	25.63	33.05	11.60	7.72	7.61	99.80

The soils employed were Coloma sand, Coloma fine sand, and Coloma sandy loam from the plots in experiment IV, the mechanical analyses of which are given in table 1. They were placed in the containers in 6-inch strata corresponding to the depth of sampling. Thus, one cylinder contained soil from 0 to 12 inches, a second, from 12 to 24 inches and a third, from 24 to 36 inches. A water-holding capacity determination was made on a 12-inch column of each stratum of the various soils. From these data were calculated the optimum moisture content to be maintained in each container during the growth of the corn.

The soil in each container was brought to its required moisture content by adding the necessary volume of water, in which was dissolved 0.75 gm. of dibasic potassium phosphate and a like amount of sodium nitrate. The penetration of the water added was hastened by attaching a strong aspirating pump to the glass tubes, which drew the water quickly and uniformly down through the entire mass of soil in each container.

The temperature of the soils was controlled by placing the containers in a large water bath in the greenhouse. The bath was provided with three motor-driven stirrers and the heat supplied by two large incandescent electric light bulbs which were controlled by an electric thermostat. Cold water coils in the bottom of the bath permitted cooling when the greenhouse temperatures became higher than that maintained in the bath. With these controls the bath was kept to within 1 degree variation of the required temperatures. A front view of the bath is shown in plate 1.

Four kernels of Golden Glow corn were planted in each cylinder on March 15 and later each container was thinned to two plants. Dixon (1) finds that best root development in young corn plants takes place at 20°C. and that as the plants develop the optimum temperature increases to about 24°C. The temperature of the bath was, therefore, maintained at 20°C. until the plants attained a height of about 6 inches and was raised to 24°C. as the plants developed. The plants grew 46 days after they appeared above the soil. During that period they attained an average height of 97 cm. On the forty-seventh day the tops of the containers were sealed over with plasticene.

The plants were considered as permanently wilted when they did not recover in a chamber at 90 per cent relative humidity and temperature of 29.3°C. (85°F.)

After permanent wilting, the soil column was removed from the containers and placed under cover where the soil was jarred free from the roots. Replicate samples of soil were taken from each container for moisture determinations.

RESULTS

The amount and distribution of rainfall at Madison for corn during July and August was good in 1918, 1921 and 1923, but poor in 1917, 1919, and 1922 (2, p. 205). Moisture conditions on the plots near Hancock were favorable in 1922, but unfavorable in 1923 (2, p. 206).

The influence of texture as a medium through which plants secured moisture under the conditions of precipitation as stated above is indicated by a comparison of the yields of corn for favorable with those for unfavorable seasons of distribution of rainfall. This comparison is graphically shown in figure 1, which indicates:

First, larger yields of corn on all classes of soil during seasons of favorable distribution than during those of poor distribution of rainfall. The open character of these soils, especially the medium and fine sand, permit them to hold, under heaviest precipitation, but little more than the optimum amount of moisture for plant growth.

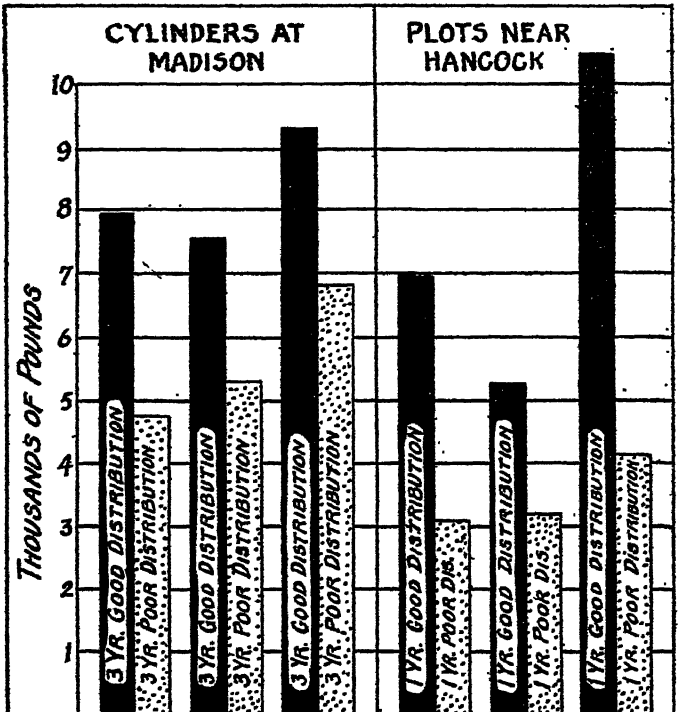

FIG. 1. AVERAGE NUMBER OF POUNDS OF AIR-DRIED CORN PRODUCED DURING SEASONS OF GOOD AND POOR DISTRIBUTION OF RAINFALL ON MEDIUM SAND, FINE SAND, AND SANDY LOAM OF THE COLOMA SERIES ON CYLINDERS AT MADISON AND ON PLOTS NEAR HANCOCK

Second, during seasons of favorable distribution of rainfall, medium sand produces larger yields than fine sand, but under conditions of poor distribution the order of production is reversed. Sandy loam, with its higher silt content and the presence of a clayey sand subsoil, is able to store moisture from the heavier rains in sufficient amounts to produce the larger yields during seasons of poor as well as those of favorable distribution.

The above facts are substantiated also by a critical study of the rainfall records for Madison and Hancock (2, p. 205, 206) whereby it is possible to group the distribution of rainfall, during the two months prior to the maturity of the crop, into three types:

Type 1 (infrequent heavy rains and light showers) includes intervals during which 50 per cent, or more, of the precipitaion occurs as isolated rains of 0.9 inch or more, or groups of rains occurring within a 3-day period, totaling 0.9 inch or over, with intervening intervals of 15 to 30 days during which occasional light showers may occur.

Type 2 (infrequent heavy rains and frequent light showers) includes intervals during which 50 per cent, or more, of the precipitation occurs largely as groups of rains within a 3-day period, totaling 0.9 inch, or more, with intervening intervals of 7 to 15 days during which occasional light rains and showers occur.

Type 3 (frequent light showers) includes intervals during which the total precipitation is uniformly distributed in individual rains or groups of light rains and showers from 0.35 to 0.9 inch *within* intervals of 7 to 15 days.

TABLE 2

The relation of soil texture to type of distribution of rainfall as determined by yields of corn

TYPE OF DISTRIBUTION	YIELDS OF CORN		
	Lowest on	Intermediate on	Highest on
Infrequent heavy rains and light showers	Medium sand	Fine sand	Sandy loam
Infrequent heavy rains and frequent light showers	Medium or fine sand	Fine, or medium sand	Sandy loam
Frequent light showers	Fine sand	Medium sand	Sandy loam

The effect of texture on the ability of the soil to supply moisture received under these types of distribution is reflected in the yields of corn produced. By arranging the textures of the soil in ascending order, as determined by the yields produced upon them, and assigning them to the type of distribution into which they fall, the arrangement as shown in table 2 is obtained.

It is apparent that under conditions of the first type of distribution, the water-holding capacity of the various soils is the determining factor in the supply of moisture to crops, for the loss by percolation from the heavy rains is proportionately greater in the medium sand than in the fine sand and also in the fine sand than in the sandy loam.

Under the conditions of the second type, the values of medium and fine sand for corn are practically equal. This indicates a resultant effect of the ability of the two soils to hold water from the heavier rains and the depth to which the water from the lighter rains penetrates.

The superiority of the medium sand over fine sand in the economic use of light rains is clearly shown under the third type of distribution, and is a result of the more rapid and deeper penetration of water from light rains into the root zone.

In spite of the fact that sandy loam utilizes the water from light rains and showers least advantageously, its greater ability to store water from the early spring rains and heavy rains during the growing season enables it more nearly to meet the water requirements of the crop.

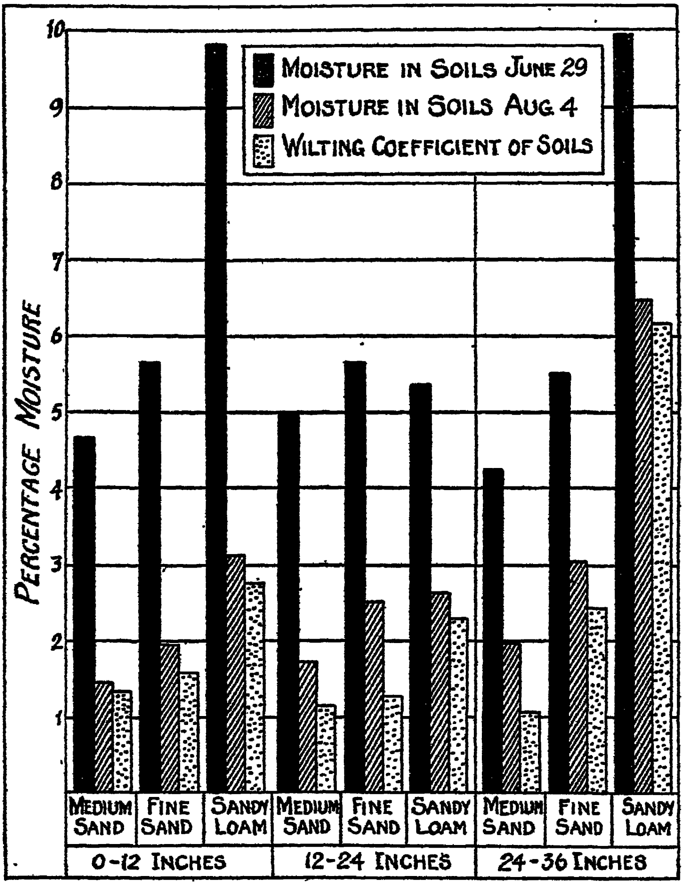

FIG. 2. MOISTURE CONTENT OF COLOMA MEDIUM SAND, COLOMA FINE SAND, AND COLOMA SANDY LOAM TO A DEPTH OF 3 FEET ON JUNE 29 AND AUGUST 4, 1923, ON PLOTS NEAR HANCOCK WITH THEIR WILTING COEFFICIENTS

The extent to which the moisture supply in sandy soil is depleted during extended periods of drought and the effect upon the corn crop was most striking in 1923 on the experimental plots near Hancock.

There was a grouping of rains on June 24, 25, and 26, totaling about 1.75

inches followed by an exceedingly dry period to August 16, with intervening showers of 0.49, 0.47 and 0.40 inch on July 6, 23, and 26 respectively (2, p. 206). Moisture determinations were made on June 29 and again on August 4. The percentages of moisture on these dates, together with their wilting coefficients, are graphically shown in figure 2.

The uniform distribution of moisture in the Coloma medium and fine sands on June 29 is evident, the variations in the three depths corresponding to the differences in mechanical composition. The distribution in the Coloma sandy loam, however, although practially equal in the first and third foot, is considerably lower in the second foot. This is brought about largely by the greater water-holding capacity and capillary pull of the soil in the first and third foot depth due to the greater amount of silt in the first foot and the higher clay content in the third foot, as shown in table 1.

A comparison of the moisture contents on August 4 with the wilting coefficients shows that these soils, under field conditions, closely approach the wilting coefficients. The leaves of the corn were badly curled on the medium sand and fine sand plots and during the warmer part of the day some curling was observed on the sandy loam plot. This condition persisted until a rain of over 1 inch occurred on August 21. No ears formed on the corn of any of the plots.

Observations made in 1920 on corn growing on these soils in cylinders at Madison, show that on July 20, following 0.57 and 0.63 inch rains of July 6 and 8, the leaves of the corn growing on medium sand and on fine sand started to curl during the middle of the day. On July 28 the corn on the medium and fine sands was quite badly wilted, but that on the sandy loam was still turgid, although not making much growth. This indicates that the sandy loam has a sufficiently high water-holding capacity to carry corn through 20 days of July weather having a mean maximum temperature of 78.5°F., a mean minimum of 59.3°F., and an average mean of 68.9°F. following a 1.2 inch rain without wilting, but that on medium sand and fine sand, wilting begins within nine or ten days.

SUMMARY

The results of a study of the effect of texture of sandy soils on moisture supply for corn during seasons of favorable and unfavorable distribution of rainfall and the proximity to which these soils approach their wilting coefficient during periods of drought are summarized as follows:

1. The favorable distribution of a given amount of precipitation during the growing season produces larger yields of corn on Coloma sand, Coloma fine sand, and Coloma sandy loam than those produced under conditions of poor distribution.

2. Rainfall is classified into three types as to distribution: First, infrequent heavy rains and light showers; second, infrequent heavy rains and frequent light showers; and third, frequent light showers.

3. Yields of corn under the first type of distribution of rainfall are highest on sandy loam, intermediate on fine sand, and lowest on medium sand. Those under the second type are

highest on sandy loam but the same for both medium and fine sand. Under conditions of the third type, however, the yields are highest on sandy loam, intermediate on medium sand, and lowest on fine sand.

4. The moisture content of the three soils, following a period of drought, closely approaches their wilting coefficient to a depth of three feet below the surface.

5. Observations made in July, 1920 show that the leaves of corn growing on medium and fine sand showed signs of wilting 12 days after a 3-day accumulated rainfall of 1.20 inches. Twenty days after the rain, the corn on these two soils was badly wilted, but that on the sandy loam was still turgid, indicating that the sandy loam has a sufficiently high water-holding capacity to carry corn without signs of wilting, through 20 days of July weather having a mean maximum temperature of 78.5°F., a minimum of 59.3°F., and an average mean of 68.9°F.

REFERENCES

(1) Dixon, J. G. 1926 Unpublished data from the Department of Pathology, University of Wisconsin.

(2) Stewart, H. W. 1926 A study of some of the factors affecting the supply of moisture to crops in sandy soils. *Soil Sci.* 21: 197–223.

(3) Whitson, A. R., and Baker, O. E. 1912 The climate of Wisconsin and its relation to agriculture. Wis. Agr. Exp. Sta. Bul. 223.

PLATE 1

Constant Temperature Bath and Corn

Fig. 1. Before wilting of corn.
Fig. 2. At wilting of corn.

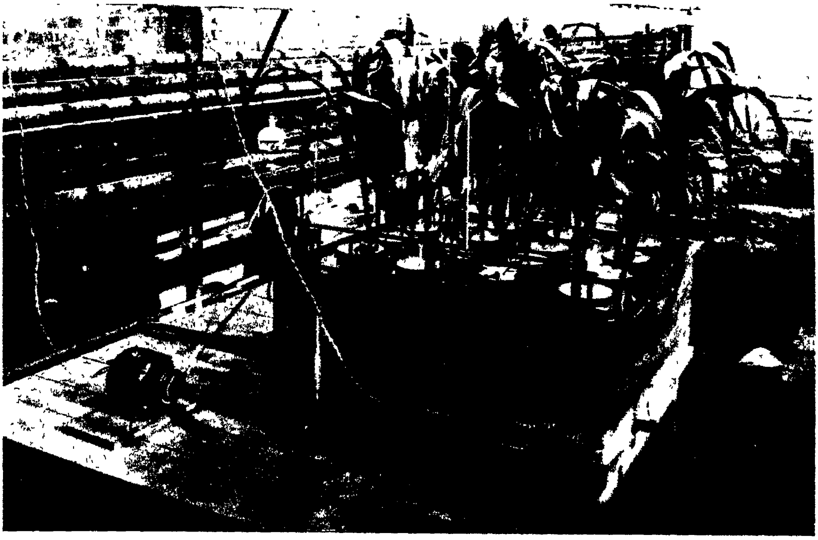

FIG. 1

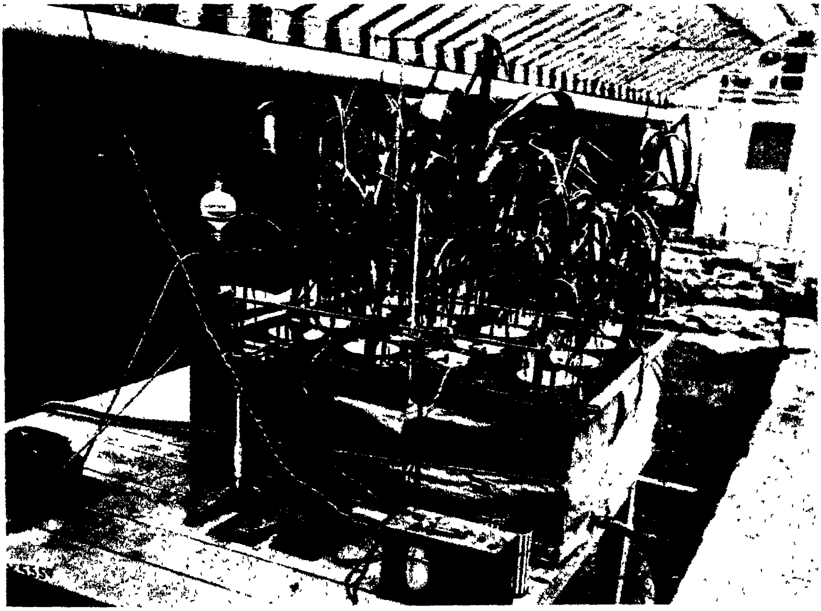

FIG. 2

PERCENTAGE OF CARBON DIOXIDE IN SOIL AIR

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During a recent meeting of the American Society of Agronomy at Washington, a discussion arose regarding the effect on photosynthesis of a possible increase in the percentage of carbon dioxide in the air as a result of decaying organic matter in the soil. The data recorded in this paper may be of some interest in this connection as they include analyses of the soil air in the presence of different amounts of organic matter and under different weather conditions. They also include the percentage of carbon dioxide in samples of air above the soil taken at the same time as the soil samples. It is therefore possible to compare the percentage of carbon dioxide in the air above the surface of the soil with a known content of carbon dioxide in the soil. The experiments were planned primarily to test the effect of carbon dioxide in the soil atmosphere on the sprouting of potatoes and on the growth of the sprouts. Besides the decay of organic matter, it was found that the high respiration of large cut seed pieces may also be a source of carbon dioxide in the soil under certain conditions.

EXPERIMENTAL PROCEDURE

The experiments covered an early crop of Irish Cobblers planted on April 28 and a late crop of McCormick potatoes planted on June 30. The beginning of the growing season for the early crop was noticeably dry, permitting good aeration of the soil during the period covered by the experiment. The weather conditions for the late crop were very different. The season was so wet for a period after planting that cultivation was not possible until July 25.

One lot of the early crop seed was planted in good potato soil but in the bottom of the furrow of one row was placed organic matter consisting of grass and green volunteer oats. A second lot was dropped in a plow furrow and heavy sod turned over on the seed. A third lot was planted next to lot 2 but the sod had been plowed in March. The late crop seed was planted in 5 rows of 50 feet each. Large tubers were cut in half and the pieces planted 12 inches apart in the row. Green alfalfa was placed in the bottom of the furrow of one row and green cabbage leaves were placed in the furrow of another row. The organic matter was covered with a little soil before the seed was dropped. The two outside rows and the middle row contained no added organic matter and were considered the check rows.

At stated intervals after planting, samples of the soil atmosphere as well as of the air above the surface were taken for gas analysis. The samples of air in the soil were taken four to five inches below the surface and included samples from between rows as well as from different points in the rows and as near the seed pieces as possible. The growth of the plants and the yield of tubers were correlated with the composition of the soil atmosphere under the different treatments.

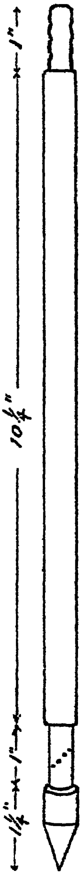

FIG. 1. SAMPLING TUBE FOR THE COLLECTION OF CARBON DIOXIDE

METHODS OF GAS ANALYSIS[1]

A special sampling tube was designed and constructed from two brass cylinders. The smaller one fits tightly inside the larger one and projects about one

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[1] The gas analyses by Hesse's method were made by J. M. Arthur, former assistant in this laboratory.

TABLE 1

Percentage of carbon dioxide in the soil atmosphere around potato hills of an early crop

Duplicate samples were taken at different points in the row. The later samples were taken under plants of different sizes.

DATE OF SAMPLING	GOOD POTATO SOIL		HEAVY SOD	
	No organic matter added	Organic matter placed in furrow	Turned on seed	Plowed in march
	CO_2 per cent	*CO_2 per cent*	*CO_2 per cent*	*CO_2 per cent*
May 1	0.038	0.24	0.06	0.07
	0.042	0.19	0.09	0.05
	0.044	0.17		
	0.038	0.13		
May 15	0.110	0.31	0.08	0.24
	0.095	0.31	0.11	0.17
May 25	0.098	0.26	0.04	0.17
	0.099	0.19	0.08	0.12
		0.17		
		0.17		
		0.30		
		0.26		
June 1		0.26	0.25	
		0.38	0.26	
		0.38	0.30	
		0.18	0.29	

TABLE 2

Percentage of carbon dioxide in the soil atmosphere and in the air above the surface—late crop of potatoes

DATE OF SAMPLING	ORGANIC MATTER PLACED IN THE FURROW		NO ORGANIC MATTER ADDED		AIR 12 INCHES ABOVE SURFACE OF THE SOIL
	Alfalfa row	Cabbage row	Control row	Between rows	
	CO_2 per cent	*CO_2 per cent*	*CO_2 per cent*	*CO_2 per cent*	*CO_2 per cent*
July 16*	6.91	2.21	5.05	1.4	0.03+
July 17†	5.31	2.97			0.03+
After cultivation					
July 26*	0.62		0.60		
August 8†	0.27	0.35	0.35		
	0.37	0.22	0.23		

* Volumetric method.

† Hesse's method.

inch at each end. One projection is grooved for a rubber tube connection and the other is drilled with 12 small holes arranged in a spiral. A sharp pointed piece of metal of the same diameter as the outer cylinder is then fitted to the end of the drilled projection. When the tube was forced into the soil the section containing the small holes was surrounded by an air space or loose soil. If the soil was very dry the holes were covered by a layer of silk but usually this was unnecessary. The sampling tube is shown in figure 1. The samples of air for the volumetric analysis were collected in 250 cc.-gas-collecting tubes by mercury displacement. The first 100 cc. of air drawn from the soil was allowed to escape in order to wash out the sampling tube and connections.

The carbon dioxide content of the late crop soil was measured volumetrically and also by Hesse's method in duplicate samples. Because of the small percentages of carbon dioxide in the early crop soil it was measured only by Hesse's method. The results of the gas analyses are shown in tables 1 and 2.

DISCUSSION OF RESULTS

The results show that the incorporation of organic matter in potato rows increased the carbon dioxide in the soil but this increase was much smaller under the early crop than under the plants of the late crop. This difference in the carbon dioxide of the soil may be attributed largely to the different weather conditions prevailing during the two experimental periods, as the amount of soil moisture would be expected to influence not only the diffusion of the gases from the soil but also the rate at which the organic matter decays. During the wet season of the late crop the percentage of carbon dioxide in the soil air of one of the rows in which organic matter had been added was as high as 6.9 per cent. Of special interest is the high percentage of carbon dioxide in the soil atmosphere of the control row to which no organic matter was added. The high respiration of the large cut seed pieces no doubt contributed a considerable amount of carbon dioxide to the 5.05 per cent total found on July 16, as the samples were taken as near as possible to the seed piece.

The late crop was cultivated for the first time on July 25. The analyses of the soil air on the following day showed that cultivation had reduced the carbon dioxide in both the organic and control rows over 90 per cent, a remarkable demonstration of the effect of cultivation on the aeration of this soil after a period of wet weather.

When the percentage of carbon dioxide in the soil air was highest, the analyses of the air 12 inches above the surface did not show any significant increase in percentage of carbon dioxide above the normal amount in air out-of-doors. If analyses of the air had been made soon after cultivation when the accumulated carbon dioxide in the soil was escaping rapidly, it is altogether possible that some increase in the air near the surface might have been observed. Lundegärdh (1) made gas analyses of the air in a well fertilized field of beets in October. He found that the content of carbon dioxide was

0.0534 to 0.284 per cent at the surface and 0.0401 to 0.0674 per cent above the leaves. From the data in this paper, however, it seems very probable that any significant increase of carbon dioxide in moving field air as a result of decaying organic matter in the soil would be very transitory and variable because of changes in weather and soil conditions.

The chief interest in the results of these experiments lies in the gas analyses rather than in the effect of the accumulated carbon dioxide on the sprouting of the tubers or on the growth of the plants when the entire growth period is considered. The plants of the early crop came up earlier in rows containing organic matter than in the control row. This was probably due to conservation of moisture by the organic matter as the season was dry and also cool up to May 24 permitting rather slow decay of the added organic matter.

The seed pieces of the late crop all sprouted but the plants in the row containing the highest percentage of carbon dioxide were only 4 inches high when those in the control row were 8 inches high. At this time the content of carbon dioxide in the soil was greatly reduced by cultivation. The row of backward plants began a vigorous growth and by the end of this growing period had practically caught up to the control plants. The early slow growth of the plants in the organic row may have been due to a narcotic effect of the accumulated carbon dioxide or it may have been due to low oxygen pressure, as analysis of the soil air showed that the oxygen at some points had been reduced to 10 per cent.

These experiments were prompted by a report of potatoes not sprouting in plowed sod during a wet season. It was thought that carbon dioxide might have accumulated in this soil to the extent of checking sprout growth. This was hardly possible, however, since these experiments show that even the high carbon dioxide content of the late crop soil did not prevent the tubers from sprouting.

REFERENCE

(1) LUNDEGÅRDH, H. 1924 Der Kreislauf der Kohlensäure in der Natur. Jena.

NITRATES AND WHEAT YIELDS AFTER CERTAIN CROPS

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An experiment with the following 3-year crop rotations[2] was begun on the experiment station farm at Lexington in 1921 (the plot numbers are given with the rotations):

1. Corn, wheat, clover
2. Corn, wheat, orchard grass
3. Tobacco, wheat, clover
4. Corn, wheat, soybeans (in rows)
5. Corn, soybeans (in rows), wheat
6. Corn, soybeans (drilled solid), wheat
7. Corn, wheat, clover as on plot 1.

Three series of plots are used so that each crop is grown each year. Wheat is grown in all the rotations. A primary purpose of the experiment is to determine the effect of the various rotations and, in particular, of the immediately preceding crops on the yield of the wheat crop. The plots are plowed for corn, tobacco, and soybeans, and disked for wheat. Soybeans are removed for hay. Corn is removed at the fodder stage. Hay crops of clover and orchard grass are removed. No farm manure, commercial fertilizer, or liming treatments have been used on the plots. The land is average, of the highly phosphatic central bluegrass land. The nitrogen content in the surface soil averages around 3200 pounds to 2 million pounds of soil. The wheat yields given in table 3 should be a fairly good indication of its productiveness.

Differences in wheat yields after the growth of certain crops, are supposed to be due, to a considerable extent, to differences brought about in nitrate content in the soil. In the bluegrass region of Kentucky, the yield of wheat is commonly known to be considerably larger after tobacco than after corn, the corn being cut for fodder. This has been found to be so in the general crops experiments on the experiment station farm. This increased yield undoubtedly is partly due to the earlier removal of the tobacco crop from the land and the better physical condition of the soil, which make better seeding of the wheat on samples of soils from crop rotation plots 1 and 3, which had been in the laboratory several days. To make the procedures alike where otherwise there would be seemingly unessential differences, the following changes were made in Harper's procedure: 100 gm. of soil, 300 cc. of water, 0.8 gm, of $CaCO_3$, and 2 gm. of $MgCO_3$ were used instead of 50 gm. soil, 250 cc. water, 0.4 gm. of $CaCO_3$, and 1 gm, of $MgCO_3$. Fifty cubic centimeter filtrate portions were evaporated on the bath. Determinations were on duplicate filtrate portions. Plot 1 soil was used only in its moist condition. Plot 3 soil was used in its moist condition and after being air-dried in the laboratory.

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[1] E. M. Johnson, assistant in agronomy, assisted in the nitrate determinations in part of this work.

[2] This experiment as well as other experiments on the station farm referred to in this paper are under the direction of George Roberts, head, and E. J. Kinney, professor of farm crops, department of agronomy.

TABLE 2

Results of a comparison of Harper's procedure and the procedure used in the work of this paper

PLOT NUMBER OF SOIL SAMPLE	PROCEDURE USED	FILTRATE USED	COLORIMETER READINGS OF STANDARD SOLUTION	COLORIMETER READINGS OF SOIL FILTRATE SOLUTION	PROPORTION RECOVERED OF STANDARD ADDED, AVERAGE OF DUPLICATE
					per cent
1	Work of this paper	Soil	40 40	69 64	
1	Harper's	Soil	40 40	78 94	
3	Work of this paper	Soil	40 40	50 50	
3	Work of this paper	Soil plus 5 cc. standard solution	40 40	23 23	93.9
3	Harper's	Soil	40 40	60 58	
3	Harper's	Soil plus 5 cc. standard solution	40 40	29 29	70.0
3 air-dry	Work of this paper	Soil	40 40	60 62	
3 air-dry	Work of this paper	Soil plus 5 cc. standard solution	40 40	26 26	88.5
3 air-dry	Harper's	Soil	40 40	90 84	
3 air-dry	Harper's	Soil plus 5 cc. standard solution	40 40	46 48	39.2

In all cases, filtrates secured by Harper's procedure were the clearer—those from the moist soils were entirely clear; that from the air-dry soil showed a

TABLE 3

Pounds of nitrates (NO_3) in 2,000,000 pounds of dry soil and wheat yields in bushels per acre

PLOT NUMBER	ROTATION	NITRATES		WHEAT	NITRATES				WHEAT	NITRATES									WHEAT	NITRATES						WHEAT	NITRATES					WHEAT
		October 28, 1921	December 21, 1921	1922	October 31, 1922	March 30, 1923	April 27, 1923	May 24, 1923	1923	October 10, 1923	November 1, 1923	December 19, 1923	April 15, 1924	May 6, 1924	May 27, 1924	June 16, 1924	July 9, 1924	Average October 10 to November 1 samplings	1924*	September 19, 1924	October 6, 1924	October 28, 1924	November 25, 1924	December 16, 1924	Average September 19 to November 25	1925	September 18, 1925	October 5, 1925	October 20, 1925	November 25, 1925	Average of the 4 samplings	1926
		lb.	*lb.*	*bu.*	*lb.*	*lb.*	*lb.*	*lb.*	*bu.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*bu.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*bu.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*bu.*
1	Corn, wheat clover	193	18	15.3	146	10.0	31.0	5	23.7	13.5	13	9.4	21.7	18.9	8.8	6.4	8.2	13.3	8.7	39.1	34.8	54.3	46.0	11.6	43.6	23.3	113.0	122	27.9	9.1	68.0	34.3
2	Corn, wheat, orchard grass....	185	22	15.7	98	13.0	7.5	5	23.3	11.4	8	9.2	14.4	18.0	5.3	5.6	7.1	9.7	6.0	19.0	18.9	25.3	35.3	11.2	24.6	21.7	37.5	62	23.6	10.1	33.3	29.0
3	Tobacco, wheat Clover........	88	30	14.0	108	13.0	7.5	5	25.3	54.0	43	29.0	27.9	22.1	7.2	7.7	6.6	48.5	15.0	17.8	35.4	27.0	51.3	14.4	32.9	32.3	332.0	249	90.5	30.8	175.6	45.7
4	Corn, wheat, soybeans (in rows)	170	21	16.3	74	9.0	7.5	5	19.7	16.0	10	15.1	21.9	38.0	6.0	7.3	7.3	13.0	11.3	18.9	23.6	31.7	36.5	11.7	27.7	22.7	47.5	49	22.6	14.7	33.5	25.0
5	Corn, soybeans (in rows) wheat	108	21	17.7	85	8.5	7.5	5	20.7	23.8	35	14.2	16.6	12.5	7.2	6.7	8.5	29.4	16.6	32.2	20.1	33.6	31.5	13.0	29.4	19.3	93.5	114	31.4	14.2	63.3	36.3
6	Corn, soybeans (solid) wheat...	127	20	21.3	52	12.0	7.5	5	17.3	29.0	32	13.6	19.8	13.0	5.1	12.2	8.7	30.5	18.0	17.2	20.2	24.2	27.1	11.1	22.2	21.0	55.5	89	27.9	13.0	46.4	31.7
7	Corn, wheat clover.........	182	20	15.0	202	10.0	7.5	5	24.7	17.1	11	12.1	17.2	15.3	4.2	7.7	9.6	14.0	10.0	32.0	32.2	36.4	39.9	11.6	35.1	24.0	123.0	75	22.9	8.0	57.2	36.7
	Subsoil of plot 3..										43	36.1	17.7	17.3	12.2	6.7	6.1			8.5	14.2	13.5	22.1	25.5			55.5	64	147.2	63.0		

* Low yields due to extremely dry weather during the preceding fall, which prevented germination of wheat until very late. Moisture conditions on plots 3, 5, and 6 were more satisfactory than on the others.

trace of color. Filtrates from the procedure used in the work of this paper had a brownish yellow tinge; that from the dry soil considerably more noticeable than the others. Examination of the residues in the dishes after evaporation on the bath showed similar differences between Harper's and the procedure used in this work. The colorimeter readings, however, showed Harper's (as above modified) to be inferior, in the case of these soils, to the procedure used in this work, giving low results whether with soil filtrates alone or soil filtrates plus standard solution and poorer agreement between duplicate determinations, as shown by the results in table 2.

Results from the nitrate determinations and the corresponding wheat yields are given in table 3. To make comparison easier, averages are given in table 4. The crops maturing in 1921 were the first in the experiment so that the nitrate determinations in the fall of 1921—the first in the nitrate study—and the wheat yields of the following year represent the second year of the experiment; and the nitrate determinations and wheat yields in the following year, the third year of the experiment or the completion of the first turn of the rotations. The nitrate result and wheat yield averages in table 4 are given for two periods, the first two years of the study representing the last two years of the first turn of the rotations, and the last three years of the study representing the second turn of the rotations. This is done because there are certain variations in both the nitrate results and the wheat yields between the two periods. It is natural to suppose these variations to be due, in part at least, to effects during the first period, incident to getting the rotations under way. The

TABLE 4

Average nitrate content in soils and average wheat yields

PLOT NUMBER	ROTATION	FIRST PERIOD		LAST PERIOD	
		Average nitrates, October 28, 1921 to October 31, 1922, per 2,000,000 lb. dry soil	Average wheat yields, 1922, 1923, per acre	Average nitrates* September, October, November, samplings, 1923, 1924, 1925, per 2,000,000 lb. dry soil	Average wheat yields, 1924, 1925, 1926, per acre
		lb.	*bu.*	*lb.*	*bu.*
1	Corn, wheat, clover	170	19.5	41.6	22.1
2	Corn, wheat, orchard grass	142	19.5	22.5	18.9
3	Tobacco, wheat, clover	98	19.7	85.7	31.0
4	Corn, wheat, soybeans (in rows)	122	18.0	24.7	19.7
5	Corn, soybeans (in rows), wheat	97	19.2	40.7	24.1
6	Corn, soybeans (drilled solid), wheat	90	19.3	33.0	23.6
7	Corn, wheat, clover	192	19.9	35.5	23.6

* Averages of the yearly averages from table 3.

results of this period, therefore should be of less value in measuring the true effects of the rotations than the results of the last period. The more important variations between the two periods and, insofar as possible, explanations for them are as follows:

In the main, the nitrates in the plots are much higher in the first than in the last period (table 4). Some allowance should be made in comparing the nitrate figures in this table for the two periods because the results in the last period are averages of samplings extending later into the fall when a seasonal decline in nitrates takes place. This, however, was only a small factor in the difference between the periods, as the results in table 3 from approximately the same time in the different years show.

The land in the rotation experiment was in red clover preceding the experiment. The clover was seeded in wheat in the spring of 1919, and a large clover growth resulted. The hay crop was removed but the heavy sod and extensive other growth were probably a factor of some importance in the generally high nitrate level during the first period.

A further probable reason for the generally high nitrate level at the October 28, 1921 sampling (table 3), was the stimulating effect on nitrification of the dry soil, which was due to a severe drought during the last half of July and the first half of August in this year. (The high nitrate level during the fall of 1925 (table 3) is to be similarly accounted for. The drought during this year was of equal length but one month later.)

At the October 28, 1921 sampling, the amount of nitrates in plots 2 and 4 was practically the same as in plots 1 and 7 (table 3). In the last period the amount was much lower in plots 2 and 4 (table 4). Plots 1, 2, 4, and 7 were plowed from the original clover sod in the spring of 1921, had grown only the crop of corn in the rotations, and there had been no chance for the rotations to affect the results of the 1921 sampling.

The amount of nitrates at the October 28, 1921 sampling in plot 3, which grew tobacco during the year, is low compared with the amounts in plots 1, 2, 4, and 7, which grew corn (table 3). This also is at variance with the comparative amounts in these plots in the last period (table 4). Plot 3 was plowed from the sod at the same time and handled in practically the same way as plots 1, 2, 4, and 7 except for the difference in crops grown during the year. The drought caused low corn yields on plots 1, 2, 4, and 7, which averaged 32.6 bushels per acre. A large growth of tobacco on plot 3, however, was made, the yield being 2095 pounds per acre. This was largely made after the drought was broken in the middle of August. Probably a limited use of nitrates by the small corn crop and an extensive use of nitrates by the large tobacco crop account for the differences in nitrates found.

In addition to whatever effects still remained from the roots, stubble, and aftergrowth of the clover on the land previous to the beginning of the experiment, the relatively large amount of nitrates in plots 1, 2, 3, and 7 at the October 31, 1922, sampling (table 3) is to be accounted for by the fact that these plots were plowed from the original clover sod after the hay crop was removed in the summer of 1920 but were reseeded to clover and orchard grass on the appropriate plots in the late summer of that year and remained in sod until the spring of 1921.

The probable explanation for the differences in nitrate content between plots 1, 3, and 7 at the October 31, 1922, sampling (table 3) is interesting. In these particular differences, the nitrates are also at variance with the results in the last period (tables 3 and 4). The corn yields on plots 1 and 7, in 1922, were 48.6 and 39.7 bushels per acre, respectively. The tobacco yield on plot 3 was 1160 pounds per acre. These are very roughly average yields for the crops and probably would be supposed to be of less importance in bringing about differences in nitrate content in the fall than would considerable differences in the preceding clover growth on the plots. The yields of clover hay on the plots during the preceding year were: plot 1, 2620 pounds (per acre); plot 3, 1960 pounds; and plot 7, 3480 pounds. If the clover growth remaining on the plots is assumed to be proportionate to the hay crops removed, the ratio between this growth and nitrate content found is practically the same with the three plots.

Nitrates on plots 5 and 6 are lower than on plots 1 and 7 in the first period (table 4) but they are about the same in the last period. There is no apparent reason for this change in relationship between the periods.

The average wheat yields during the first period (table 4) are fairly uniform on the various plots and, on the whole, lower than during the last period. Averaging the results smoothes out some differences apparent in the individual years in the first period (table 3), but even here the comparative uniformity is more significant than such differences as do exist. Effects from the rotations, seemingly, have not yet appeared.

There appears to be no relationship between nitrate content as determined, and wheat yields during this first period, unless the wheat yields on the tobacco-wheat-clover rotation (plot 3) were no larger than on the corn-wheat-clover rotations (plots 1 and 7) because of the relatively low nitrate content in the tobacco-wheat-clover plot (table 4).

The results of the last 3-year period, representing the second turn of the rotations, will now be considered. Nitrates were higher on the tobacco-wheat-clover plot than on the corn-wheat-clover plots during the fall of 1923, somewhat lower during 1924, for which there is no apparent explanation, and much higher during 1925 (table 3). Wheat yields were higher on the former plot during all three years. The averages of both nitrate content and wheat yields were much higher in the tobacco-wheat-clover rotation than in the corn-wheat-clover rotation (table 4).

The substitution of orchard grass for clover (plot 2, compared with plots 1 and 7, table 4 last three-year period) decidedly reduced nitrates, and lowered the yield of wheat. The average acre yields of the hay crops removed from these plots for the 5-year period (1921 to 1925) were 3624 pounds of clover hay and 1860 pounds of orchard-grass hay.

The substitution of soybeans (in rows and cultivated) for clover (plot 4, compared with plots 1 and 7, table 4) also decidedly reduced nitrates. The nitrates in plot 4 were about the same as in the corn-wheat-orchard-grass plot 2. Wheat yields correlate very well with the nitrates found (table 4) being lower on plot 4 than on plots 1 and 7 and about the same as on plot 2.

It is interesting to compare plot 4 with plot 5 (table 4). Nitrates are appreciably higher in plot 5, after soybeans, than in plot 4, after corn. Wheat yields are likewise higher on plot 5, the difference being 4.4 bushels an acre. The yields of corn and soybeans are higher on plot 4 than on plot 5, the average 1921 to 1925 crop yields per acre being as follows: soybeans, plot 4, 4408 pounds; plot 5, 3888 pounds; corn, plot 4, 57.7 bushels; plot 5, 47.8 bushels.

In other areas which adjoin each other on the station farm, wheat after soybeans in a four-year rotation of corn, soybeans, wheat and clover, as an average of 12 crops in the period 1913 to 1925, has yielded 4.8 bushels more per acre than after corn in a 3-year rotation of corn, wheat, and clover (4). The corn and clover yields are both slightly higher in the 3-year rotation, probably because of greater natural soil productiveness; therefore the increased wheat yields here also seemingly should be credited to the superiority of soybeans over corn as a preceding crop.

The rotation on plot 6 is corn, soybeans, wheat, but differs from that on plot 5 in that the soybeans on plot 6 are drilled solid and have not been cultivated except during 1925 when cultivated once with a smoothing harrow. The soybeans on plot 5 are in rows and have been cultivated three or four times. In the fall of 1925 nitrates were appreciably and consistently higher on plot 5 than on plot 6 (table 3). Differences in the other years are small and probably of no significance. Wheat yields (table 3) were considerably higher on plot 5 than on plot 6, in 1926 (corresponding with 1925 nitrate results) and the differences were small in the other years. The acre yields of soybeans on the two plots were, as an average of the 5 crops, 1921 to 1925, 3888 pounds on plot 5 and 3960 pounds on plot 6.

In general there is a definite relationship between nitrates as determined and wheat yields during the last 3-year period, higher wheat yields correlating with higher nitrates.

Data giving production of nitrates under wheat during the spring, in addition to during the preceding fall, probably would show a closer relationship between nitrates and wheat yields than when restricted to the fall alone. With this in view, plots were sampled and nitrates determined in the year following seeding of the wheat in 1923 and 1924. The results (table 3) on the whole show a low and uniform nitrate content in the plots, suggesting that the crop fed very closely on nitrates which prevented differences in nitrate production from appearing; and therefore are useless for the purpose intended.

Probably this would not happen under all soil conditions. Whiting and Schoonover (6) report one year's results with the determination of nitrates in the fall and the following year on plots at Urbana, Illinois, when in wheat following soybeans. On many of the plots, nitrates were found to be as high during the spring and early summer as during the preceding fall. It is worth noting in the work of Whiting and Schoonover that nitrates in the plots receiving phosphate treatments were considerably lower than in the other plots, suggesting that the close feeding of the wheat on nitrates found in the Lexington rotation plots was associated with the high phosphorus soil content and the relatively lower nitrogen content.

To study the effect of certain crops on nitrate production during the following season in the absence of the effect of a growing crop, the following work was done. Crops as shown in table 5 were grown on small plots, about 1/40 acre. During the fall after the crops were removed and during the following spring and summer, these plots were kept fallow without plowing. The plots were sampled and nitrates determined as on the crop rotation plots. The crops affected nitrate production somewhat in the fall. Nitrates were lower after hemp and to a less extent after corn, than after the other crops. They were higher after soybeans than after tobacco. The results perhaps show smaller production of nitrates after corn and hemp than after the other crops during the first part of the following season. Nitrates increased on all plots throughout the season and, in the main, were at about the same level. At the date of the last sampling, on August 8, the average amount of nitrates in the plots was 235 pounds to 2 million pounds of soil.

TABLE 5

Nitrates (NO_3) in 2,000,000 pounds of dry soil in ground kept fallow after certain crops

CROP	1923			1924					
	October 10	November 1	December 19	April 15	May 6	May 27	June 16	July 9	August 8
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Tobacco	31.2	44	29.6	40.1	68.5	114.0	107.4	190.3	217.8
Hemp	15.0	15	15.2	40.0	45.4	76.0	97.6	200.5	235.3
Oats	33.6	39	28.8	42.8	71.3	90.0	118.6	192.3	242.7
Soybeans	63.0	63	18.3	45.3	70.7	99.3	95.2	189.4	235.9
Corn	23.3	14	13.7	27.8	50.5	91.8	100.8	190.7	246.9
Subsurface of corn plot 7 to 14-inch layer		12	18.8	13.3	29.4	54.6	49.2	72.0	240.2

Welton and Morris (5) report results from nitrate determinations in soils under and immediately after certain crops and discuss the relation between nitrates found and yields of immediately following wheat crops. They state that apparently there is a close relationship between nitrates and wheat yields. Their investigations were particularly on the effect on wheat yields of high nitrates after potatoes, but the effects after corn, oats, wheat, clover, and soybeans, were also studied. Their results do not in all cases show positive relationship between nitrate content and wheat yields. Yields after soybeans in the rotations compared were better than after corn, whereas nitrate content was invariably found somewhat lower after soybeans than after corn. They also report results of nitrate determinations during one fall, in corn and tobacco land. The average of all determinations showed 20 parts of nitrates per million of dry soil in the tobacco land, and 10 parts per million in the corn land.

Samples were taken from the 7- to 14-inch layer and nitrates determined in the corn plot in the fallow plots. It will be noted (table 5) that the amount of nitrates in this layer during 1924 was around half of that in the surface soil. The nitrates in this layer greatly increased, however, from July 9 to August 8, becoming practically equal to the amount in the surface soil. Records of the local weather bureau station located one mile from the plots show a rainfall of 1.50 inches on July 12. As this came when the ground was already fairly moist, some removal of nitrates from surface to under soil no doubt occurred. At no other time was precipitation sufficient for leaching to occur. It seems that removal of nitrates from surface to under soil could account for only the smaller part of the increase in the under soil, particularly in view of the large increase in nitrates in the surface soil during the same interval. Seemingly the rest is to be accounted for by actual production of nitrates in this layer.

Nitrates also have been determined in the 7- to 14-inch depth, in the tobacco-wheat-clover plot 3, beginning with the November 1, 1923, sampling. These results are given in table 3. In general nitrates have increased in this layer during the fall, coincidently with a decrease in the surface soil. Here it seems clear that the leaching effect of rainfall on the surface soil is the main factor. The large increase in nitrates, 64 to 147.2 pounds, in the 7- to 14-inch depth, between the October 5 and October 20, 1925, samplings, will be noted. Nitrates in the surface soil decreased from 249 to 90.5 pounds during the same interval. There were 4.36 inches of rainfall between the two dates; 2.66 inches fell on October 16.

SUMMARY

Results are reported of nitrate determinations extending over five years on soil samples from the plots of a crop rotation experiment on the Lexington experiment station farm. A primary purpose of this experiment is to determine the effect of the various rotations and, in particular, of the immediately preceding crop on yields of the wheat crop. This crop follows corn, tobacco, and soybeans. There are also other differences in the rotations. Nitrates were determined mainly in the fall when wheat was seeded, to ascertain the relationship between nitrate production and wheat yields. The averages of the results of the last three years of the study show a fairly close relationship between nitrates and wheat yields. This is considered to indicate the probable relationship in the future. However, considering the results by years and particularly including the first two years of the study, the relationship is shown to be only very general and to have no correlation at numerous points.

REFERENCES

(1) Harper, H. J. 1924 The accurate determination of nitrates in soils. *Jour. Indus. Engin. Chem.* 16: 180-183.

(2) Noyes, H. A. 1919 Accurate determination of soil nitrates by phenol disulfonic acid method. *Jour. Indus. Engin. Chem.* 11: 213–218.

(3) Potter, R. S., and Snyder, R. S. 1915 The determination of nitrates in soil. *Jour. Indus. Engin. Chem.* 7: 863, 864.

(4) Roberts, G., Kinney, E. J., and Freeman, J. F. 1926 Field experiments on soils and crops. Ky. Agr. Exp. Sta. Bul. 272.

(5) Welton, F. H., and Morris, V. H. 1924 Yields of wheat following potatoes and the relation of nitrates in the soils to these. *Jour. Amer. Soc. Agron.* 16: 519–534.

(6) Whiting, A. L., and Schoonover, W. R. 1920 Nitrate production in field soils in Illinois. *Ill. Agr. Exp. Sta. Bul.* 225: 40–42.

THE VARIABLE OCCURRENCE OF NITRATES IN SOILS

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Results from determinations of nitrates in the plots of a crop rotation experiment have been reported (1). In the last year of this work, it was noticed that the amounts of nitrates found in certain plots changed considerably in relation to each other between consecutive dates of sampling. The most pronounced instance of this was in the results from plots 6 and 7 at the September 18 and October 5, 1925, reading. The nitrate nitrogen found in these plots at the two dates, expressed as pounds in 2 million pounds of dry soil, was:

	PLOT 6	PLOT 7
September 18	12.5	27.8
October 5	20.1	17.0

It seemed unlikely that these changes in nitrates actually occurred in the plots. The more probable explanation was that the method of sampling used did not in all cases accurately represent the nitrates in the plots.

The plots in the rotation experiment are 21 by 103.7 feet in size (1/20 acre). Samples were taken from two lines of the full length of a plot, and equidistant from each other and from the sides of the plots. On plots in corn and tobacco these lines were midway between rows. Nine cores to a depth of 7 inches were taken with a soil auger in each line. A composite sample was made by mixing together the 18 cores. The cores of any sampling were always located at some distance from the cores of preceding sampling. The number of cores taken was at least as large as the average for an equal area taken for the determination of nitrates in field soils and it was thought would represent the nitrates in the soils sufficiently for the purposes of the work.

When it was noticed in the fall of 1925 that apparently the samples taken did not in all cases accurately represent the nitrates in the soils, some work was done to get information on the variability of nitrates in the soil of these plots and in certain other areas on the experiment station farm.

It is thought that the results of this work, although limited in extent, might be of interest as confirming more extensive work (1, 2) showing the wide variability of nitrate occurrence in apparently uniform soils, and because seemingly the importance of this variability is likely to be underestimated in sampling soils for nitrate determination.

On October 20, 1925, plots 1 and 3 were sampled twice, with the following results: plot 1, first sampling, 6.2 (pounds nitrate nitrogen per 2 million pounds dry soil); second sampling, 6.4; plot 3, first sampling, 24.3; second sampling, 16.6. Plot 1 was in corn during the summer. Plot 3 was in tobacco.

On October 23, plot 3 was sampled three times; first in the usual manner, second in the usual manner with the cores midway between the cores of the first sampling, and third the usual number of cores taken at the usual distance apart but located directly in the two adjoining rows on the south of the two middles from which the first and second samplings were obtained. The nitrate nitrogen found in the three samplings was: first, 16.6 (pounds per 2 million pounds of dry soil); second, 24.3; third, 12.5. Plot 5 (in soybeans during the summer, in rows and cultivated) was sampled twice on this date, with the cores of one sampling midway between the cores of the other. The nitrate nitrogen found in the two samples was 6.8 and 7.6, respectively.

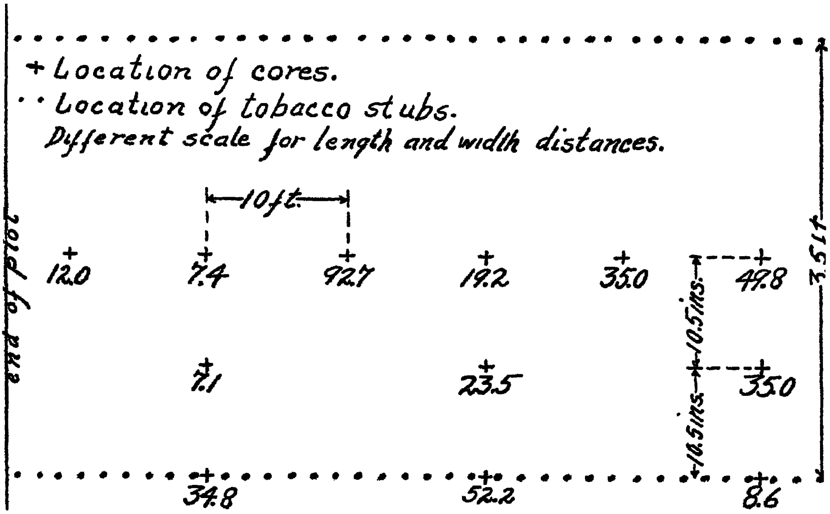

FIG. 1. VARIABILITY IN OCCURRENCE OF NITRATES IN SURFACE SOIL OF A SMALL AREA IN CROP ROTATION PLOT 3 SAMPLED OCTOBER 28, 1925

Figures show pounds of nitrate nitrogen in respective cores per 2,000,000 pounds of dry soil

On October 28, 1925, 12 cores of surface soil were taken from plot 3, 6 from the central middle, and 6 from one of the adjoining rows and nitrates determined in the individual cores. The location of the cores and the nitrate nitrogen found are shown in figure 1. This plot has received no treatments of lime materials, commercial fertilizers, or farm manure for a period of at least 10 years.

On November 10, 1925, 5 cores of surface soil were taken from the center of a middle and 5 directly in one of the adjoining rows of another tobacco plot on the experiment station farm located some distance from the crop rotation plots. Samples were also taken from the 7 to 14-inch depth of the 5 middle cores. The location of the cores and the nitrate nitrogen found in the

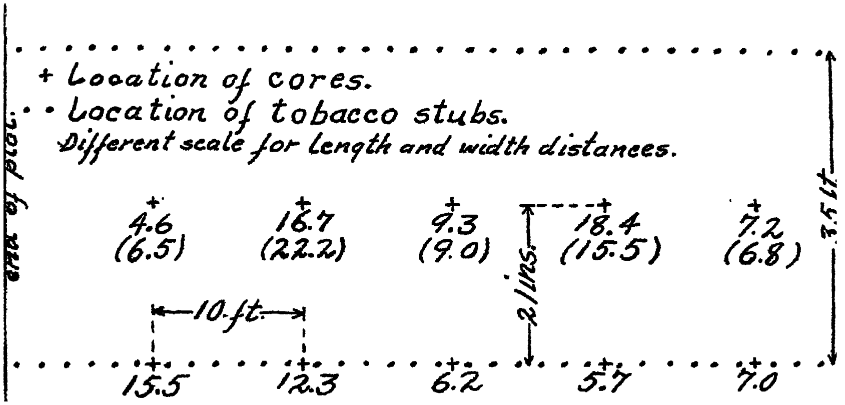

FIG. 2. VARIABILITY IN OCCURRENCE OF NITRATES IN A SMALL AREA IN A TOBACCO VARIETY PLOT, SAMPLED NOVEMBER 10, 1925

Figures show pounds of nitrate nitrogen in respective cores per 2,000,000 pounds of dry soil; figures in parentheses are from 7- to 14-inch depth.

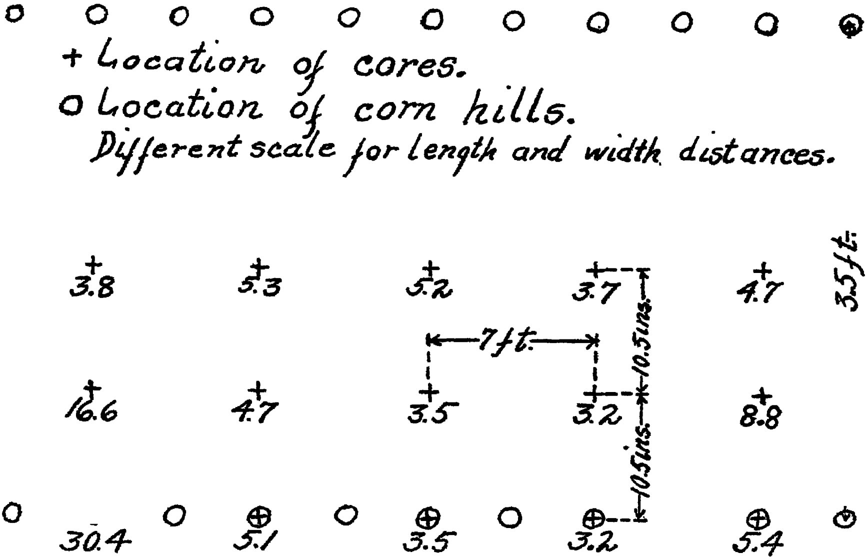

FIG. 3. VARIABILITY IN OCCURRENCE OF NITRATES IN THE SOIL OF THE SURFACE FOOT OF A SMALL AREA IN A PLOT OF A MANURE EXPERIMENT IN CORN DURING THE SUMMER

Figures show pounds of nitrate nitrogen in respective cores per 2,000,000 pounds of dry soil

individual samples are shown in figure 2. This plot was in corn in 1924 and received 10 tons of farm manure per acre. No commercial fertilizer or liming treatments have been used.

On November 13, 1925, 15 cores were taken from the surface foot of soil in a middle and one of the adjoining rows of a corn plot in a manure experiment on the experiment station farm. A 3-year rotation of corn, wheat, and clover is used on the plot. Farm manure is applied at the rate of 10 tons per acre on the ground after plowing for corn. No liming or commercial fertilizer treatments have been used in the last 15 years. The location of the cores and the nitrate nitrogen found in the individual cores are shown in figure 3. When the sampling was done, preparation of the ground for the seeding of wheat had fairly well leveled the plot and moved most of the corn stubs. Enough were in place, however, to make possible fairly accurate location of hills.

All three of the areas in which the nitrates were determined in individual cores were quite uniform as far as was shown by field examination.

The data are not extensive enough to warrant statistical treatment or much discussion. It will be noted that, excepting the two high figures in the corn plot in the manure experiment, variability in nitrate occurrence was greatest in tobacco plot 3 in the crop rotation plots, intermediate in the other tobacco plot, and least in the corn plot in the manure experiment. This order corresponds with the order of average amounts of nitrates present in the three areas, the greatest variability being associated with the greatest amount of nitrates present. This is also borne out by the results from the duplication of samplings on plots 1, 3, and 5 in the crop rotation experiment, where the lack of agreement between duplicate samples was much greater in the case of plot 3 with the larger amount of nitrates than with the other two plots. The results also seem to show that variation was likely to be as great in short distances as in relatively much greater distances. On all three areas sampled for nitrate determination in individual cores, the distance between cores in a lengthwise direction of the middles was several times that of the distance between cores in a crosswise direction of the middles. Variations in nitrate nitrogen, however, on the whole were just as great between consecutive crosswise samples as between consecutive lengthwise samples (figs. 1, 2, 3). Apparently variability in nitrate occurrence was not correlated in any way with possible differences brought about by cultivation of the corn or tobacco crops on the areas, or with different residual effects from stubs and roots of the tobacco or corn plants such as might be expected to obtain between samples of soil directly in the row or hill and at varying distances away.

REFERENCES

(1) Karraker, P. E. 1927 Nitrates and wheat yields after certain crops. *Soil Sci. 24:* 247–258.

(2) Prince, A. L. 1923. Variability of nitrates and soil nitrogen. *Soil Sci.* 15: 395–405.

(3) Waynick, D. D. 1918. Variability in soils and its significance to past and future soil investigations: 1. A statistical study of nitrification in soils. *Univ. Cal. Pubs Agr. Sci.* 3: 243–270.

SOME REACTIONS BETWEEN MONO-CALCIUM PHOSPHATE AND SOILS[1]

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Considerable work has been done on the insolubility of phosphorus of the soil but the greater part of the work has been hothouse and field plot work interpreted in terms of crop response. This kind of work is valuable but does not determine the several factors and their influence upon the solubility of the phosphorus of the soil. In order to obtain fundamental results, it becomes necessary to isolate and study separately the several soil factors involved. The influence of the soil base on the solubility of $CaH_4(PO_4)_2$ can best be observed, perhaps, by titrating $CaH_4(PO_4)_2$ and these soil bases in pure form in neutral distilled water. When three equivalents each of CaO, MgO, and $CaCO_3$ were titrated separately with $CaH_4(PO_4)_2$ three strikingly different reaction curves were obtained. In the titration of CaO with $CaH_4(PO_4)_2$ the reaction changed from pH 12.2 to below pH 6 with the addition of less than one equivalent of $CaH_4(PO_4)_2$ to three equivalents of CaO. MgO gave a curve somewhat similar in form to the CaO curve but of much shorter range in pH. The reaction of $CaCO_3$ titration changed very little until after the addition of two equivalents of $CaH_4(PO_4)_2$, at which point the pH decreased rapidly.

Different compounds were also formed in these titrations. Amorphous $Ca_3(PO_4)_2$ occurred at points 1 to 10 inclusive on the horizontal scale of the graph (fig. 1) and crystalline $CaHPO_4$ occurred at points 2 to 10 inclusive in the titration of CaO. The tendency of the chemical reaction at the addition of one equivalent of $CaH_4(PO_4)_2$ appears to be as follows, the $Ca(OH)_2$ being present in excess,

$$3Ca(OH)_2 + CaH_4(PO_4)_2 = Ca_3(PO_4)_2 + Ca(OH)_2 + 4H_2O$$

With the addition of two equivalents of $CaH_4(PO_4)_2$ the reaction probably is more complete as indicated in the following equation.

$$6Ca(OH)_2 + 2CaH_4(PO_4)_2 = Ca_3(PO_4)_2 + 2CaHPO_4 + 3Ca(OH)_2 + 6H_2O$$

but this reaction, does not progress to completion, for at each point a small amount of the transition product, $CaHPO_4$, was found, which seemed to indicate that the reaction may be more as follows,

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[1] Taken from thesis presented at Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

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$$3Ca(OH)_2 + 2CaH_4(PO_4)_2 = Ca_3(PO_4)_2 + 2CaHPO_4 + 6H_2O$$

If a third equivalent of $CaH_4(PO_4)_2$ had been added the reaction probably would have been as follows,

$$3Ca(OH)_2 + 3CaH_4(PO_4)_2 = 6CaHPO_4 + 6H_2O$$

The conditions existing in these titrations appear to be contrary to the accepted idea, in that there exists at the point of the first equivalent of $CaH_4(PO_4)_2$ (fig. 1) solid $Ca_3(PO_4)_2$, solid $CaHPO_4$, and solid $Ca(OH)_2$ in a liquid medium with a reaction below pH 6, indicating the presence of a substance which ionizes, giving free H ions. This reaction is approximately the reaction of $CaHPO_4$. These equations, however, do not show the exact conditions existing because these chemical reactions do not proceed in gram-molecular quantities but in much smaller proportional quantities. The base, $Ca(OH)_2$, is present in a large excess at the beginning of the titration and an excess is found as a solid sediment even below the neutral point.

A somewhat similar condition is found to exist in flasks 6, table 1, in which, $Ca_3(PO_4)_2$ exists as a precipitate in a liquid medium with reactions of pH 6.5, pH 6.3, and pH 5.5. respectively. The conditions are partially represented by the equation,

$$6CaH_4(PO_4)_2 + 9Ca(OH)_2 = 4Ca_3(PO_4)_2 + 2CaHPO_4 + CaH_4(PO_4)_2 + 18H_2O$$

for the titration equivalent to 100 pounds of acid phosphate per acre. In the other two more concentrated titrations, more $Ca_3(PO_4)_2$ and $CaH_4(PO_4)_2$ and about the same amount of $CaHPO_4$ is present, the latter being in solution in each case. $Ca_3(PO_4)_2$ was also found as a solid medium of pH 6.5 in the titration of $CaHPO_4$ with $Ca(OH)_2$ as shown in table 2. Even with a proportion of $Ca(OH)_2$ to $CaHPO_4$ of 5 to 4 the reaction was practically neutral, pH 6.9.

$CaHPO_4$ is considered a transition product, its stability depending on the reaction of the medium in which it occurs. It was, however, the principal compound formed in the titration of $CaCO_3$, being found as a precipitate at points 4 to 16, inclusive, in increasing amounts. Amorphous $Ca_3(PO_4)_2$ could not be detected at any point in the titration. The quantity of $CaCO_3$ remaining in the bottoms of containers decreased from points 1 to 8, no $CaCO_3$ being observed beyond point 8. The tendency of the chemical reactions are represented by the following equations:

1st equiv., $3CaCO_3 + CaH_4(PO_4)_2 = 2CaHPO_4 + Ca(HCO_3)_2 + CaCO_3$
2nd equiv., $3CaCO_3 + 2CaH_4(PO_4)_2 = 4CaHPO_4 + Ca(HCO_3)_2 + H_2O + CO_2$
3rd equiv., $3CaCO_3 + 3CaH_4(PO_4)_2 = 6CaHPO_4 + 3H_2O + 3CO_2$

In the titration of MgO the chemical reactions are more complex because of the introduction of another base-ion, magnesium, the phosphorus compounds of which are more soluble than the calcium phosphates. All of the products formed in this titration were soluble in the quantities formed, as no precipitates appeared. It is probable that the di-basic phosphates principally were formed, with the chemical reaction proceeding according to the following equation, at the point of the addition of three equivalents of $CaH_4(PO_4)_2$:

$$Mg(OH)_2 + CaH_4(PO_4)_2 = MgHPO_4 + CaHPO_4 + 2H_2O$$

TABLE 1

Solubility of phosphorus in titration of $CaH_4(PO_4)_2$ with $Ca(OH)_2$

Concentrations equivalent to 100, 200, and 300 pounds acid phosphate per acre. Three inches containing 12 per cent moisture. Time; 14 days.

FLASK NUMBER	RATIO: $\frac{Ca(OH)_2}{CaH_4(PO_4)_2}$	100			200			300		
		pH	Precipitate	Added P found in solution	pH	Precipitate	Added P found in solution	pH	Precipitate	Added P found in solution
				per cent			*per cent*			*per cent*
X	0	3.60	....	100.0	3.50	....	100.0	3.40	....	100.0
1	¼	5.57	....	100.0	6.35	....	94.4	6.25	dt	89.4
2	½	....	....	99.5	5.99	d	77.0	5.89	t	76.8
3	¾	6.31	d	70.1	5.92	(d?) t	64.1	5.79	t	64.2
4	1	6.23	dt	57.4	5.96	t	58.5	5.82	t	51.8
5	1¼	6.31	dt	40.1	6.09	t	40.4	5.96	t	38.0
6	1½	6.50	t	28.0	6.31	t	26.8	5.45	t	25.2
7	1¾	6.79	t	15.6	6.52	t	15.7	6.37	t	13.4
8	2	....	t	8.6	6.88	t	6.2	6.48	t	3.4
9	2¼	....	t	1.3	7.20	t	6.2	7.13	t	0.6
10	2½	....	t	0.8	8.34	t	0.9	8.68	t	0.1
11	2¾	8.36	t	0.5	8.78	t	0.9	9.11	t	Trace
12	3	8.60	t	0.2	9.01	t	0.7	9.21	t	Trace
13	3¼	8.65	t	Trace	9.19	t	Trace	9.52	t	Trace
14	3½	9.02	t	Trace	9.43	t	Not analyzed	9.55	t	Not analyzed
15	3¾	9.06	t	Trace	9.58	t	Not analyzed	9.60	t	Not analyzed
16	4	9.21	t	Trace	9.58	t	Not analyzed	9.75	t	Not analyzed

d = precipitate of $CaHPO_4$ present.
t = precipitate of $Ca_3(PO_4)_2$ present.

Freshly prepared $Fe(OH)_3$ and $Al(OH)_3$ had comparatively little effect on the pH of the mixture when titrated against $CaH_4(PO_4)_2$, even when the $Fe(OH)_3$:$CaH_4(PO_4)_2$ ratio was very wide. However, the $Fe(OH)_3$ exhibited a greater influence on the solubility of the phosphorus than did $Al(OH)_3$. According to the simple equation,

$$Fe(OH)_3 + CaH_4(PO_4)_2 = FePO_4 + CaHPO_4 + 3H_2O$$

the quantity of $Fe(OH)_3$ used in flask 1 (table 3) exceeds that required to complete the equation. The extent of this chemical reaction was not determined, for the solubility of $FePO_4$ permits the reaction to be completed and no phosphorus to be lost from solution as $FePO_4$.

TABLE 2

Titration of $CaHPO_4$ with $Ca(OH)_2$ solution

0.04038 gm. $CaHPO_4$ in solution in each flask. Total volume; 250 cc. Time; 14 days.

FLASK NUMBER	RATIO: $\frac{Ca(OH)_2}{CaHPO_4}$	pH	PRECIPITATES	Added P found in solution
				per cent
X	0	.....	.....	100.0
1	¼	6.49	d	73.3
2	½	6.52	t	45.4
3	¾	6.69	t	33.0
4	1	6.86	t	9.5
5	1¼	6.89	t	1.8
6	1½	7.23	t	1.4
7	1¾	8.57	t	1.8
8	2	.....	t	Trace
9	2¼	10.28	t	Trace

d = precipitate of $CaHPO_4$ present.
t = precipitate of $Ca_3(PO_4)_2$ present.

TABLE 3

Titration of $CaH_4(PO_4)_2$ with $Fe(OH)_3$ and $Al(OH)_3$

0.02958 gm. $CaH_4(PO_4)_2$ per flask. Total volume; 250 cc. Time; 14 days.

FLASK NUMBER	RATIO: $\frac{Me(OH)_3}{CaH_4(PO_4)_2}$	$Fe(OH)_3$		$Al(OH)_3$	
		pH	Added P found in solution	pH	Added P found in solution
			per cent		*per cent*
0	0	3.40	100.0	3.40	100.0
1	4	6.02	40.6	5.44	82.5
2	8	6.88	19.0	5.99	68.2
3	16	7.00	1.8	6.53	51.4
4	32	....	Trace	7.37	22.9
5	48	7.19	Trace	7.41	12.0
6	64	....	Trace	7.51	6.0
7	80	....	Not analyzed	7.29	2.3
8	160	7.66	Not analyzed	7.30	0.9
9	240	8.34	Not analyzed	7.42	0.7
10	320	8.37	Not analyzed	....	Trace
11	400	....	Not analyzed	....	Trace

This, however, is not possible with $AlPO_4$, which is very insoluble, although 82 per cent of the added phosphorus remained in solution (table 3). The chemical reaction represented by the equation,

$$Al(OH)_3 + CaH_4(PO_4)_2 = AlPO_4 + CaHPO_4 + 3H_2O$$

therefore, is incomplete. The results of these experiments do not substantiate the general opinion that aluminum renders phosphorus highly insoluble in an acid-soil medium.

The results of titrations of $CaH_4(PO_4)_2$ with KOH in several concentrations (3), with $Ca(OH)_2$ in different concentrations (table 1), and with $Al(OH)_3$ and $Fe(OH)_3$ (table 3), clearly indicate that the change in reaction of the solution of $CaH_4(PO_4)_2$ from acid to alkaline is accompanied by the change from the mono- to the di- and tri-phosphates, which are less soluble. The data obtained in these titrations also point to pH 5.6 or slightly above as the region of $CaHPO_4$-formation.

The reaction at which the most marked change occurs in phosphorus solubility is not the same in all of the titrations, but it approaches the neutral point. When a mixture of $Ca(OH)_2 + \frac{1}{2}Fe(OH)_3$ was titrated with $CaH_4(PO_4)_2$ (table 4) the change in phosphorus solubility was very sharp, occurring between pH 5.6 and pH 6.7. A marked change also occurred between pH 6.8 and pH 7 in the titration of $CaH_4(PO_4)_2$ with $Fe(OH)_3$ alone (table 3), but with $(AlOH)_3$ (table 3) this marked change in phosphorus solubility was not obtained. $Ca(OH)_2$ (table 1) caused a rapid but rather uniform decrease in phosphorus solubility, which was decidedly low at the neutral or slightly alkaline reaction. In a mixture of $CaH_4(PO_4)_2$ and $Fe(OH)_3$ or of $CaH_4(PO_4)_2$ and $Al(OH)_3$ titrated with $Ca(OH)_2$ (table 5) a uniform and rapid decrease in phosphorus solubility occurred.

TABLE 4

Solubility of phosphorus in titration of $[Ca(OH)_2 + \frac{1}{2}Fe(OH)_3]$ *with* $CaH_4(PO_4)_2$

25.5 cc. saturated solution $Ca(OH)_2$ per flask. Time; 14 days.

FLASK NUMBER	RATIO: $\frac{CaH_4(PO_4)_2}{Ca(OH)_2}$	pH	Added P found in solution
			per cent
1	$\frac{1}{4}$	6.93	2.8
2	$\frac{1}{2}$	6.72	3.9
3	$\frac{3}{4}$	5.63	25.3
4	1	5.63	40.6
5	$1\frac{1}{4}$	5.55	54.8
6	$1\frac{1}{2}$	5.61	60.2
7	$1\frac{3}{4}$	5.56	60.4
8	2	5.46	66.4
9	$2\frac{1}{4}$	5.39	70.5
10	$2\frac{1}{2}$	5.34	73.0

Phosphorus in a soil medium is subjected to the influence of the compounds of calcium, magnesium, sodium, potassium, iron, and aluminum, and the reaction of the soil together with many other soil factors. It is a recognized fact that soluble phosphorus when added to a calcareous soil reverts to the less soluble forms. It is shown by these experiments that the reverting action does not progress to the formation of insoluble $Ca_3(PO_4)_2$, but to $CaHPO_4$. In the presence of magnesium, sodium, and potassium a certain amount of the phosphorus is combined with these elements, tending to increase the quantity of soluble phosphorus. The supply of iron and aluminum in most soils far exceeds that required to convert all of the soil phosphorus into $FePO_4$ and $AlPO_4$, respectively, and since $CaH_4(PO_4)_2$, when titrated with $Al(OH)_3$, is not made insoluble by the formation of $AlPO_4$ it can not be considered that the phosphorus is thus made insoluble in the soil in the presence of other compounds with which both aluminum and phosphorus may react. The reaction of the soil, which is in reality only a measure of the balance of the H^+- and OH^--ions, is important in the consideration of phosphorus solubility since any substance introduced which changes the medium from an acid to an alkaline reaction, is accompanied by a decrease in the phosphorus in solution, because of the reversion of the phosphorus. The reaction, however, can not be taken as an index to phosphorus solubility, for in some soils even as acid as pH 4.5 (2), the soluble phosphorus is extremely low, though soluble phosphorus has been added to the soil. In view of the foregoing data, the great insolubility of phosphorus can not be due to the soil reaction or to the content of iron and aluminum but must be due, at least in part, to other yet unknown factors.

TABLE 5

Solubility of Phosphorus in Titration of $[CaH_4(PO_4)_2 + \frac{1}{2}Fe(OH)_3]$ *and of* $[CaH_4(PO_4)_2 + \frac{1}{2}Al(OH)_3]$ *with* $Ca(OH)_2$

0.11832 gm. $CaH_4(PO_4)_2$ per flask (equiv. to 200 pounds acid phosphate per acre). Total volume; 250 cc. Time; 14 days.

FLASK NUMBER	RATIO: $\frac{Ca(OH)_2}{CaH_4(PO_4)_2}$	$[CaH_4(PO_4)_2 + \frac{1}{2}Fe(OH)_3]$		$[CaH_4(PO_4)_2 + Al(OH)_3]$	
		pH	Added P found in solution	pH	Added P found in solution
			per cent		*per cent*
X	No $Fe(OH)_3$	3.50	100.0	3.50	100.0
0	0	5.33	86.9	4.26	97.2
1	$\frac{1}{4}$	6.24	81.0	6.49	81.0
2	$\frac{1}{2}$	6.24	63.5	6.57	60.1
3	$\frac{3}{4}$	6.00	51.3	6.47	43.9
4	1	6.10	37.0	6.78	32.3
5	$1\frac{1}{4}$	6.20	26.3	6.78	17.8
6	$1\frac{1}{2}$	6.49	16.0	7.52	13.4
7	$1\frac{3}{4}$	6.81	6.1	7.35	2.8
8	2	6.95	1.2	....	.....
9	$2\frac{1}{4}$	7.57	0.4	7.69	2.3
10	$2\frac{1}{2}$	8.25	Not analyzed	8.57	1.1
11	$2\frac{3}{4}$	8.77	Not analyzed	8.94	1.1
12	3	8.96	Not analyzed	....	0.7
13	$3\frac{1}{4}$	....	Not analyzed	....	0.4
14	$3\frac{1}{2}$	....	0.2	....	0.5
15	$3\frac{3}{4}$	....	0.2	....	Trace
16	4	....	.....	....	Trace

SUMMARY

In the titration of $CaH_4(PO_4)_2$ with CaO, both $CaHPO_4$ and $Ca_3(PO_4)_2$ are formed, but only $CaHPO_4$ is formed in the titration with $CaCO_3$.

$Fe(OH)_3$ was much more active in making the phosphorus insoluble than was the $Al(OH)_3$.

The data obtained in these experiments do not substantiate the general belief that aluminum renders phosphorus highly insoluble in an acid soil medium.

The region of formation for $CaHPO_4$ is at pH 5.6 or slightly above.

In general a change from acid to alkaline reaction is accompanied by a rapid decrease in phosphorus solubility.

REFERENCES

(1) Breazeale, J. P., and Burgess, P. S. 1926 The availability of phosphates in calcareous or alkaline soils. *Aris. Agr. Exp. Sta. Tech. Bul.* 10: 209–237.

(2) Spurway, C. H. 1925 Some factors influencing the solubility of phosphorus in soil-acid phosphate mixtures. *Soil Sci.* 19: 399–405.

(3) Spurway, C. H. 1926 The effect of the nature of the exchangeable bases upon the retention of anions by soils. *Jour. Amer. Soc. Agron.* 18: 501.

(4) Wendt, G. L., and Clarke, A. H. 1923 An electrometric study of the neutralization of phosphoric acid by lime water. *Jour. Amer. Chem. Soc.* 45: 881–887.

THE DETERMINATION OF SOIL COLLOIDS

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Many papers have been written during the last few years dealing with the determination of the amount of colloidal material in soils. The methods proposed differ widely, and include the absorption of gases and dyes, the heat of wetting, and the total exchangeable bases. These methods recognize the fact that colloids obtained from different soils differ in their properties, so that it is necessary to prepare a standard for each soil by isolating some of its own colloid by the supercentrifuge. An objection to this procedure is that it assumes that this small proportion of the colloid isolated is representative of the whole, and Anderson (1) has shown that this is very far from accurate. Similarly, an attempt made here to use the imbibitional water (volume moisture equivalent *minus* xylene-equivalent) as an index to colloid content was defeated at the outset by the observation that the colloidal material separated in three stages gave quite different values; namely, 229, 193, and 171. Nothing less than the isolation of the *whole* of the colloidal material for the preparation of the standard would avoid this objection. Two years ago, the author expressed the view (5) that, excluding organic colloidal material, the colloid content was identical with the clay content as determined by mechanical analysis. This view was put forward to explain the fact that a clay separate on being passed repeatedly through the supercentrifuge continues to yield "ultra clay" until, as far as can be ascertained, the whole of the fraction less than 2μ in diameter is obtained in this form. This appears to be a direct proof of the identity of ordinary clay with "ultra" or colloid clay, but comparison with the clay content of soils with their colloid content, as determined by one of the methods mentioned above, has shown that discrepancies frequently occur. Thus Gile and others (4) refer to a group of 30 soils which averaged 23 per cent of colloid as determined by the method of water adsorption, but contained only 19 per cent of clay as found by mechanical analysis.

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[1] Since this communication was despatched, the April number of SOIL SCIENCE has been received containing Dr. Bouyoucos' paper on the hydrometer method of determining soil colloids (*Soil Sci.* 23 (1927): 319–331). This is, of course, a rapid approximate method for determining the clay together with a portion of the silt fractions. There is a theoretical difficulty in that the depth of the center of the hydrometer bulb below the surface (as seen from the photograph) will vary from about 5 to 15 cm., so that to estimate the concentration of particles of a fixed range, the time of settling should vary from 7 to 22 minutes. This could be calculated when the probable equilibrium position of the hydrometer had become apparent.

As a method for the determination of soil colloids, it is open to the objection that it determines silt as well as clay, and it is not justifiable to include silt as a soil colloid.

An opportunity occurrred to test this point further, through the kindness of Mr. F. W. Parker who sent the author samples of the soils providing the data in table 1 of his paper on base exchange in soil colloids (7) which contains the results of determinations of the colloidal material in soils by three different methods. These soils were subjected to mechanical analysis, and the results together with those taken from Mr. Parker's paper, are given in table 1 divided into two groups according to whether the colloid content was or was not in fair agreement with the clay content.

TABLE 1

Results of the mechanical analysis of certain soils

SOIL	COLLOID CONTENT BY METHOD OF			MEAN	MECHANICAL ANALYSIS	
	Replacable base	Water absorption	Heat of wetting		Clay	Silt
					per cent	*per cent*
Orangeburg	6.3	5.8	7.4	6.5	7.4	4.3
Greensville	14.0	14.4	19.5	16.0	16.4	4.8
De Kalb	5.5	6.1	6.1	5.9	5.4	13.4
Hollywood	52.1	28.5	51.7	43.1	36.7	23.6
Wooster	24.7	21.4	26.8	24.3	23.9	38.7
Susquehanna	65.5	55.5	55.6	58.9	53.2	13.1
Michigan	38.8	31.3	38.8	36.3	36.3	39.0
Gray	18.1	27.6	17.7	21.1	13.4	26.0
Mean	28.1	23.8	27.9	26.5	24.1	20.4
Cecil	32.3	32.4	47.1	37.3	24.7	8.1
Clarksville	15.0	10.6	12.4	12.7	1.9	19.7
Saginaw	38.1	34.6	40.5	37.7	18.4	22.3
Oktibbeha	74.4	87.7	72.2	78.1	49.7	20.0
Mean	39.9	41.3	43.0	41.4	23.7	17.5

The table shows that in every case where there is an important discrepancy, the content of colloid is larger than that of the clay. The question immediately suggests: are the properties forming the basis of the methods for determining the former, as has been frequently assumed, peculiar to the colloid portion of the soil? The assumption is based on very little experimental evidence. Anderson (1) found that quartz, orthoclase, hornblend, and muscovite, ground to pass a 130-mesh sieve[3] gave negligible heats of wetting, but this fineness corresponds only roughly to that of the sand fraction, as silt (less than 0.02 mm.) would require a 600-mesh sieve to separate if from sand.

Bouyoucos (2) gives 8.8 calories per gram as the heat of wetting of silica, which may be compared with 11.94, the mean value for 8 soil colloids given by the same author (3). Powdered silica also possesses considerable absorbing power for water vapor.

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[3] It is assumed that the 130-mesh sieve has apertures $\frac{1}{280}$ of an inch wide.

Another property shared by colloid and non-colloid material is that of a high water-holding capacity, which is best measured by the imbibitional water. This has been shown in a recent publication (6) to be possessed in a marked degree by silt fractions entirely devoid of very small particles, and of the properties of plasticity and shrinkage. The same remarks apply to powdered silica.

Non-colloidal bodies are known to possess base-exchange properties. The literature abounds in patents for the preparation of water-softening materials of the permutite class by subjecting clay to a temperature at which (according to Bouyoucos and others) the colloid properties completely disappear. It has also been found in this laboratory, that separated and washed silt fractions have considerable base-exchange properties, two samples having been found to contain 0.2 and 0.5 per cent of replaceable calcium, respectively.

Unless, therefore, the term "colloid" in soil or ceramic science is to be stretched to include bodies devoid of plasticity or shrinkage and incapable of forming stable suspensions, it is impossible to avoid the conclusion that none of the above methods for the estimation of soil colloids is satisfactory.

Thus, a soil which, because of its deficiency in clay, was unsuitable for a particular agricultural proposition, would not be improved by a dressing of charcoal, although this would certainly raise its power of dye-absorption.

The most important properties of a soil which are due to inorganic colloids are known when the amount and nature of the clay fraction are known. The study of the clay fraction as a whole instead of the "ultra clay" (for the preparation of which few laboratories possess the facilities) will give in a simpler and more accurate form, the information required for agricultural or ceramic work.

The choice of a property to characterize the clay fraction is difficult, for whereas the different properties examined show on the whole an excellent correlation, serious exceptions are not uncommon. Until these are explained, it would probably be better to choose a property directly connected with characters of importance in the field. The Atterberg plasticity number would be of great value if a simple method for its determination could be worked out. Meanwhile, either the chemical composition of the clay (as indicated by its silica-alumina ratio) or any of the properties referred to in this paper, give information as to the nature of the clay, which is sufficient in the majority of cases.

With regard to opinions as to the amount of colloidal material present, the swing of the pendulum has been extreme. It is a long way from the older views of Schloesing, that only a small part of the clay is colloidal, to those of the present day in which more colloid than clay is said to be present. A determination of the clay content itself is the reasonable outcome of these extreme views.

SUMMARY

The determinations of the amount of soil colloids by heat of wetting, absorption methods, or base-exchange, usually give results higher than those for the clay content as determined by mechanical analysis. The above properties are, however, possessed by bodies such as silica, silt, and "permutite" which, from the point of view of soil and ceramic science, cannot be termed colloids. At present, the inorganic soil colloid content may be taken as synonymous with the clay content.

REFERENCES

(1) ANDERSON, M. S. 1924 The heat of wetting of soil colloids. *Jour. Agr. Res.* 28: 927.

(2) BOUYOUCOS, G. J. 1918 Relationship between the unfree water and the heat of wetting of soils and its significance. Mich. Agr. Exp. Sta. Tech. Bul. 42.

(3) BOUYOUCOS, G. J. 1925 The heat of wetting as a new means of estimating the colloidal material in soils. *Soil Sci.* 19: 153–162.

(4) GILE, P. L. ET AL. 1924 Estimation of colloidal material in soils by adsorption. U. S. Dep. Agr. Bul. 1193.

(5) JOSEPH, A. F. 1925 Clays as soil colloids. *Soil Sci.* 20: 89–94.

(6) JOSEPH, A. F. 1927 The moisture equivalent of heavy soils. *Jour. Agr. Sci.* 17: 12.

(7) PARKER, F. W., AND PATE, W. W. 1926 Base exchange in soil colloids. *Jour. Amer. Soc. Agron.* 18: 470.

THE COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL: I. METHODS OF QUANTITATIVE ANALYSIS OF PLANT MATERIALS[1]

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INTRODUCTORY

In the great majority of investigations dealing with the decomposition of organic materials in the soil or by pure or mixed cultures of microörganisms, the formation or accumulation of only one of the final products of the reaction, such as carbon dioxide or ammonia, is commonly measured. In some cases, the disappearance either of one of the initial constituents of the organic matter, such as sugar, pentosan, or cellulose, or of the total organic matter itself is determined. Such methods of procedure may supply information upon certain specific transformations of some of the more common or more important constituents of the organic matter, but they are far from giving a complete picture of the complex processes taking place in the soil to which are added natural organic materials, in the form of plant stubble, green manures, and stable manures. Since the organic matter commonly added to the soil consists of more than one definite chemical constituent and the products of the reactions carried out by the soil microflora and microfauna are numerous, the decomposition of one constituent of the organic matter and the various reactions involved and the products formed cannot be studied separately, but the transformations of the various other constituents of the organic matter must also be considered. The measure of the rate of change of a single ingredient of the complex organic material may be sufficient for the purpose of following the course of the particular change taking place. To obtain definite information on the complexity of the changes which result when organic matter is added to the soil, to gain some insight into the mechanism of the processes, and especially to learn the nature of the residual substances which resist decomposition by microörganisms and which contribute to the so-called soil "humus," it is essential to determine the changes which the various constituents of the plant materials undergo in the soil when acted upon by microorganisms.

It is not at all necessary to carry out a complete, detailed analysis of the composition of the plant materials which are usually introduced into the soil, to be able to determine more definitely the mechanism of their decomposition. A proximate quantitative analysis which would account for the most important constituents of the plant material is sufficient for this purpose. Such a proximate analysis has been proposed for wood by Dore (3, 4), Schorger (7), Schwalbe (8) and others. For the study of the composition of plant materials added to the soil and of the nature of decomposition processes, these methods have to be variously modified, for three reasons: first, because of the difference in the nature of the materials used; secondly, because the soil has to be used frequently as the medium for analysis, and the presence of a considerable amount will greatly influence the selection of the methods to be used in analyzing a small quantity of organic matter added; and thirdly, because of the difference in the very nature of the problem, which involves also a study of the composition of new organic materials, synthesized by the soil microörganisms.

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The chemical methods commonly employed in foodstuff analysis are altogether unsatisfactory for this purpose, since those methods are based more upon the digestibility of the materials than upon their exact chemical nature. Digestibility of an organic substance in the system of an animal results in a group of processes altogether different from the transformation of the same organic substance added to a normal cultivated soil. The terms "crude fiber" and "nitrogen-free extractives" may be quite satisfactory for food analysis but their use in a study of the nature of soil organic matter or the transformation of natural organic matter added to the soil would tend to render unintelligible the very processes that are under investigation. These and other considerations account for lack of sufficient knowledge concerning the nature of soil organic matter or soil "humus" (10).

EXPERIMENTAL

The following constituents of the natural organic materials are most important in influencing the rapidity of decomposition of organic matter added to the soil. They allow a determination of the causes for the slow or rapid liberation of the nitrogen of the organic matter in an available form, and of the influence of the carbohydrates present in the plant upon the transformation of the nitrogen complexes. The amount and nature of the residual material left in the soil as a result of the decomposition of the plant materials added can also be measured. This material becomes a part of the soil organic matter and is commonly referred to as "humus." These major plant constituents can be grouped conveniently as follows:

1. *Ether-soluble fraction.* The fats and waxes soluble in ether are decomposed only slowly by microörganisms. They may accumulate in the soil and may even influence the rapidity of decomposition of the other plant constituents (11). A determination of these is, therefore, of importance. Frequently benzene or an alcohol-benzene mixture is used for extracting this group of substances. The alcohol-soluble materials have not been determined in this

system of analysis, since alcohol extracts from plant material certain sugars and amino compounds, which are also water-soluble and which undergo rapid decomposition when introduced into the soil. Alcohol also extracts certain lignins, which resist decomposition and which are otherwise determined in the "total lignin" and in the "alkali-soluble" fractions.

2. *Water-soluble fraction* contains those ingredients of the natural organic materials which are the first to undergo decomposition when acted upon by microörganisms; the greater the content of water-soluble materials in the plant residues, the more rapidly will decomposition set in and be completed. Cold water extracts the simple carbohydrates and their derivatives, various amino acids and peptides, and soluble minerals. Hot water extracts the starches, to some extent the pectins, certain hexosans, and various nitrogen compounds.

3. *Cellulose and hemicelluloses* decompose much more slowly than the water-soluble fraction, but they decompose readily and completely (5, 11). They form the largest group of constituents of natural organic matter of plant origin added to the soil. Since these polysaccharides are free from nitrogen, the rapidity of their decomposition will depend on the amount of nitrogen in the plant material or on the available nitrogen present in the soil.

4. *Lignins* decompose in the soil only very slowly and contribute, therefore, largely to the organic matter which accumulates in the soil and which is resistant to decomposition (so-called "humus"). A knowledge of the concentration of this fraction is very essential. Furthermore, it has been shown elsewhere (11) that not only do lignins decompose only very slowly, but their presence in the natural plant materials hinders somewhat the rapid decomposition of the celluloses with which they are combined in the form of ligno-celluloses.

5. *Proteins and their derivatives.* A knowledge of the total nitrogen content, of the nature of the protein compounds, and of the relation between the available carbon and nitrogen in the organic matter added to the soil, is of prime importance in our understanding of the availability of the nitrogen in organic residues.

6. *Ash.* The amount and nature of minerals in the organic matter is frequently of great importance in influencing the decomposition of the plant material added to the soil.

Various natural organic substances were analyzed by these methods, both in the presence and in the absence of a large excess of soil; the results were found to be rather satisfactory, with the exception of the lignin determinations. This substance or group of substances is usually obtained by treating a natural organic material with strong sulfuric (72 per cent) or fuming hydrocyloric (42 per cent) acid, or a mixture of sulfuric and hydrochloric acids. The part of the natural organic material which is free from fats, waxes, and ash, and which is not acted upon by the acid, is considered as lignin. Lignin is considered to be a single chemical complex, the composition of which is fairly well known. When the analysis, however, has to be carried out in soil, in which considerably less than 1 per cent organic matter is frequently present, the determination of the residual lignin by treatment with acids is out of the question, because 90 to 98 per cent of mineral soils will also remain unacted upon by the strong acids. To obviate this difficulty, the analysis of lignin in the soil can be based upon its ability to dissolve in alkalies. Most of the lignin is brought into solution only after continuous treatment at very high pressures and temperatures (6). At low temperatures and pressures, and even at 15 pounds pressure in the autoclave, only 50 to 60 per cent of the lignin may dissolve in alkalies. This phenomenon has to be kept in mind in applying the various methods of analysis to organic matter present in soil.

For the following investigations five organic materials were selected:

1. Wheat straw, obtained after harvesting the mature crop and threshing out the grain; the straw itself was finely ground and included both the stems and leaves. 2. Ground stems of saw grass (*Cladium effusum*). 3. Ground roots (including the lower part of the stems) of saw grass; these two samples were supplied by Mr. Hunter of the Everglades substation, Florida, to whom the authors are indebted for the courtesy. 4. Ground soybeans (soybean meal); as the plants were harvested just before the pods began to form, the material represents a plant substance rich in nitrogen, obtained during the active stage of growth. 5. Young rye seedlings; these were grown in pots in the greenhouse, for 6 weeks, until the plants reached a height of 12 to 15 inches; the moisture content of these plants was 82.8 per cent; the plants were dried at 65°C. and then ground.

ANALYTICAL METHODS

Samples of the materials were always dried at 100°C. to constant weight and the results obtained in the following analyses are reported on an oven-dry basis:

Ether extract. Five-gram portions of the material are extracted for 24 hours with cold ether in a closed flask.[2] The solvent is removed and the organic material again extracted twice with ether. The combined solvent is evaporated to dryness and the residual material dried, in weighing bottles, to a constant weight at 100°C. The results thus obtained were found to check very closely with the loss in weight of plant substance, as a result of extraction.

Cold-water extract. The residue from the ether extraction is treated with 150 cc. of distilled water and allowed to stand in the cold for 24 hours. The presence of traces of ether, which serves as a disinfectant, prevents the development of microörganisms in the aqueous extract. The extract is filtered through filter paper, and the residual organic matter washed several times with water. The filtered aqueous extract is made up to volume and divided into three portions: one-half is evaporated to dryness in platinum dishes, then weighed and ignited, giving the total water-soluble portion and the water-soluble ash; one-fourth is used for total nitrogen determination, by the Kjeldahl method; and one-fourth is used for the determination of reducing sugars, by the Bertrand method. In some instances, especially in the analysis of decomposing material, only one-quarter of the extract is used for total organic matter and ash, and the remaining quarter for ammonia (by distillation with MgO or by the aeration method) and nitrate determinations. The residual plant material, from which the water-soluble portion has been removed, is then used directly for the alkali extraction. In some cases the material is extracted with boiling water, for 30 minutes; this brings into solution the starches and certain other hexosans (especially in microbial protoplasm), some pectins, and certain nitrogenous compounds. The hot water extract is analyzed in the same manner as the cold water extract, except that one-quarter of the solution is boiled with 2 per cent hydrochloric acid, before the sugar determination is made.

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[2] It was found later that the yields thus obtained are somewhat lower than those obtained by extraction in a soxhlet for 12 to 24 hours. The latter method of extraction is to be preferred and was used in the subsequent studies.

Alkali extract. One-hundred-cubic-centimeter portions of a 5.0 per cent solution of sodium hydroxide are added to the residues from the aqueous extraction and heated in the autoclave for 30 minutes at 15 pounds pressure. About 50 cc. of cold distilled water is then added to each flask and the extract filtered through dried and weighed filter papers. Another 50-cc. portion of the same alkali solution is then added to the residue of each preparation, which has been removed carefully from the paper, and the extraction is repeated in the same manner. Filtration is repeated through the same filter paper. The residue is washed with water, with dilute acetic acid, and again with water, then dried and weighed. The difference between the weights of the residue after the cold water and after the alkali extractions gives the amount of material soluble in 5 per cent sodium hydroxide solution. In the presence of soil, this portion is found by evaporating, in silica dishes, an aliquot portion of the solution previously neutralized with HCl solution, then drying, weighing, igniting, and weighing. The alkali extract is treated with sufficient hydrochloric acid to give an excess of 2 per cent acid in solution; this solution is then boiled for 30 minutes and the precipitate filtered off upon weighed papers. The precipitate is washed with water, dried and weighed, and analyzed for total nitrogen. The weight of the precipitate, from which the protein content is subtracted (nitrogen content × 6.25), represents the alkali-soluble lignin of the preparation. The amount of lignin thus obtained is only a definite part of the total lignin present in the plant material, amounting to not more than 50 to 60 per cent of the total lignin obtained by treatment with acids ("acid-lignin"). The filtrate from the lignin is analyzed for total nitrogen and for total reducing sugar. This sugar represents some of the hemicelluloses (including some of the pentosans and the starches) which are soluble in alkalies and which are hydrolized by 2 per cent hydrochloric acid at boiling temperature.

Treatment with 2 per cent sulfuric acid. The residue from the alkali extract is then treated with 100 cc. of a 2 per cent sulfuric acid solution, at boiling temperature, for 30 minutes. The residue is washed with water, dried, and weighed. The extract is used for sugar determination. This sugar represents another part of the hemicellulose of the plant material.

Cellulose. A definite part of the residue from the acid extract is treated with an ammoniacal copper solution (modification of Schweizer's reagent), to extract the cellulose, which is then precipitated with alcohol, washed with hydrochloric acid and distilled water, dilute alkali solution, water, dilute acid, water, alcohol and ether; the precipitate is then dried, weighed, ignited and weighed again, according to the method suggested by Charpentier (2). This procedure is to be preferred to the chlorine and sulfur dioxide treatments, because of the ease of manipulation. It was also found to give at least as reliable results as the method used by Bengtsson (1), whereby the lignins are removed with sodium bisulfite and hydrochloric acid solution.

Lignins. Two-gram portions of the original material are treated, in glass-stoppered containers, with ether, and, after the removal of the ether extract, with a mixture of 10 cc. of an 18 per cent hydrochloric acid solution and 50 cc. of a 72 per cent sulfuric acid solution, as suggested by Schwalbe (9). The reacting mixture is allowed to stand for at least 30 minutes and is then transferred with 300 cc. of water to Erlenmeyer flasks and boiled for 30 minutes. The lignin is filtered off upon weighed paper, washed with water, dried, weighed, and ignited. The loss in weight may be taken as lignin, although, especially in the case of substances rich in nitrogen, the nitrogen content of the lignin may be considerable and should be accounted for.

Pentosans are determined by the ordinary phloroglucide method.

TABLE 1

General analysis of plant materials on a moisture-free basis

MATERIAL	WHEAT STRAW	SAW-GRASS STEMS	SAW-GRASS ROOTS	SOYBEAN MEAL (GROUND SOYBEAN PLANT)	YOUNG RYE PLANTS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Ether extract	1.10	1.14	0.90	3.80	2.35
Organic matter, soluble in cold water	5.57	6.87	5.21	22.09	29.54
Lignin (ash-free, protein-free)	21.60	29.09	30.90	13.84	10.61
Cellulose	39.10	28.31	30.74	28.53	17.84
Pentosans	26.35	21.45	20.75	11.08	12.67
Protein (insoluble in cold water)	2.10	7.19	3.81	11.04	12.26
Ash	3.53	3.89	3.57	9.14	12.55
Total	99.35	97.94	95.88	99.52	97.82

Proteins, insoluble in cold water, are determined by subtracting the water-soluble nitrogen from the total nitrogen, then multiplying the difference by 6.25.

Table 1 gives the proximate analysis of five different plant products.

The alcohol extract is not reported in this analysis, since it represents substances already reported under the different groups. For example, alcohol extracted from the saw-grass stems 3.88 per cent of the material and from the saw-grass roots, 2.32 per cent. This extract, upon the removal of the alcohol, was found to consist of 62.2 per cent lignin, when determined by the hydrochloric-sulfuric acid method. The remaining 37.8 per cent of the alcohol-soluble fraction could be partly accounted for by the soluble sugars and nitrogen compounds. In other words, since part of the lignin and certain nitrogen compounds and sugars are soluble in alcohol, the inclusion of this fraction is not justified.

The results obtained by analyzing the water-soluble fraction for reducing sugars, soluble nitrogen, and soluble minerals (as ash) are given in table 2. Only about a half of the fraction is thus accounted for, since no attempt has been made to determine the soluble organic acids, glucosides, starches, pectins, and other hemicelluloses and their derivatives.

The results obtained by treating the preparations (after removing the ether and water-soluble fractions) with 5 per cent sodium hydroxide, under pressure, are given in table 3. Practically two-thirds of the extract is accounted for. The widest discrepancy is found in the case of the wheat straw, largely because of the difference between the high pentosan content (26.35 per cent) in the straw and the comparatively low yield of reducing sugars in the extract. The unaccounted-for portion of the alkali solution may consist of degradation products of celluloses and lignins, as well as of some soluble forms of these substances (*pro* and *hemi* forms).

TABLE 2

Analyses of water-soluble fraction, in per cent of original material

MATERIAL	WHEAT STRAW	SAW-GRASS STEMS	SAW-GRASS ROOTS	SOYBEAN MEAL	YOUNG RYE PLANTS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Reducing sugar	1.12	1.52	0.40	4.08	6.78
Nitrogen, as protein	0.38	0.66	0.35	2.35	4.90
Water-soluble minerals	1.00	1.66	1.20	4.00	9.54
Total accounted for	2.50	3.84	1.95	10.43	21.22
Total found	5.57	6.87	5.21	22.09	29.54

TABLE 3

Analysis of the alkali-soluble fraction, in per cent of original material

MATERIAL	WHEAT STRAW	SAW-GRASS STEMS	SAW-GRASS ROOTS	SOYBEAN MEAL	YOUNG RYE PLANTS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Alkali-soluble lignin	10.08	11.95	13.44	2.71	4.49
Nitrogen, as protein	0.68	2.30	1.28	5.75	8.81
Hemicellulose, as reducing sugar	13.00	18.77	19.77	2.55	8.45
Total accounted for	23.76	33.02	34.49	11.01	21.75
Total extract found	40.52	50.20	41.70	17.66	32.45

To demonstrate whether the lignin obtained by extracting the material with alkali and precipitating with an excess of acid (on boiling) is the same as the acid lignin obtained by the action of concentrated acids, the alkali lignin was treated with a mixture of hydrochloric and sulfuric acids. Between 90.2 and 93.3 per cent of the alkali lignin was recovered as acid lignin. In other words, although only a part of the lignin, amounting to about 40 to 45 per cent of the acid lignin, is soluble in 5 per cent sodium hydroxide at 15 pounds pressure, this alkali lignin is practically identical in its reaction with the acid lignin.

Table 1 shows that, by this method of analysis, practically all of the constituents of natural organic materials, especially those of plant origin can be accounted for. This is especially true for the wheat straw, the soybean meal, and the rye plants, representing three of the most common organic materials usually added to the soil. The results for the saw grass fall short by 2.06 per cent in the case of the stems and by 4.12 per cent in the case of roots. This discrepancy can readily be accounted for, when the results for the pentosan determinations are compared with the results obtained by determining the reducing sugars in the alkali extract (neutralized with hydrochloric acid and boiled in the presence of an excess of acid) and in the 2 per cent sulfuric acid extract. In the case of the saw-grass stems, the sum of the reducing sugars thus obtained is 22.51 per cent, and in the case of the roots 25.05 per cent, whereas the corresponding results of the pentosan determinations are 21.45 and 20.75 per cent. In other words, the determination of the reducing sugars in the alkali and acid extractions gave 1.06 per cent, in the case of the stems, and 4.30 per cent, in the case of the roots, more than the pentosan determinations, indicating that this plant contains other hemicelluloses (galactans, mannans, etc.), which are not accounted for by the pentosans.

The analyses of the alkali-soluble fraction are especially illuminating in regard to the lignin determinations. Between 40 and 50 per cent of the lignin, obtained by direct treatment with concentrated acids, is found in the alkali extract of wheat straw and saw grass; however, not more than 20 per cent of the total lignin is found in the alkali extract in the case of the soybean meal. Whether this is due to differences in the nature of the lignin or to the influence of the high protein content upon lignin extraction with alkali (the alkali extract of soybean meal precipitated with acids and boiled in the presence of 2 per cent acid is rich in nitrogen and when the protein and ash contents are subtracted, the amount of lignin left is inconsiderable) remains to be seen.

REFERENCES

(1) BENGTSSON, N. 1925 Bestämning av Inkrustered cellulosa i jord. Meddel. 279, Centralanst. Försöksv. Jordbruksområdet (Sweden,. Bakt. Avd. 37.

(2) CHARPENTIER, C. A. G. 1921 Studien über den Einflusz des Rindvieh-und Pferdestallmistes auf die Zersetzung der Zellulose in der Ackererde. Diss. Helsingfors.

(3) DORE, W. H. 1920 The proximate analysis of coniferous woods. *Jour. Indus. Engin. Chem.* 12: 476–480.

(4) DORE, W. H. 1920 The proximate analysis of hardwood: Studies on Quercus agrifolia. *Jour. Indus. Engin. Chem.* 12: 984.

(5) HEUKELEKIAN, H., AND WAKSMAN, S. A. 1925 Carbon and nitrogen transformations in the decomposition of cellulose by filamentous fungi. *Jour. Biol. Chem.* 66: 323–342.

(6) MEHTA, M. M. 1925 Biochemical and histological studies on lignin: its physiological significance and its estimation in timbers. *Biochem. Jour.* 19: 958–978.

(7) SCHORGER, A. W. 1917. The chemistry of wood: I. Methods and results of analysis of some American species. *Jour. Indus. Engin. Chem.* 9: 556–560, 561–565.

(8) SCHWALBE, C. G. 1920 Die chemische Untersuchung pflanzlicher Rohstoffe und der daraus abgeschiedenen Zellstoffe. Papier-Zeitung. Berlin.

(9) SCHWALBE, H. 1925 Eine neue Methode zur Bestimmung des Lignins. *Papier Fabrik.* 23: 174–177.

(10) WAKSMAN, S. A. 1926 The origin and nature of the soil organic matter or soil "humus": I. Introductory and historical. *Soil Sci.* 22: 123–162.

(11) WAKSMAN, S. A., and Tenney, F. G. 1926 On the origin and nature of the soil organic matter or soil "humus": IV. The decomposition of the various ingredients of straw and of alfalfa meal by mixed and pure cultures of microörganisms. *Soil Sci.* 22: 395–406.

A RÉSUMÉ OF THE PROBLEM OF NITROGEN LOSSES THROUGH DENITRIFICATION

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The loss of gaseous nitrogen from nitrogenous compounds at any stage in the natural cycle is of interest to the agriculturist and sanitarian alike. In the voluminous literature on the subject, justifiable borrowing of experimental data from one field by the other is common; unfortunately though, the borrowed data are occasionally used without a just appreciation of the technical pitfalls confronting the experimenter. Among agriculturists it is generally conceded that loss of gaseous nitrogen under any but very abnormal field conditions is entirely negligible, though contributions now and then indicate disagreement over details of lesser practical importance. Likewise the sanitary engineer has largely ceased to worry about the nitrogen content of his sludge, since difficulties in obtaining a marketable fertilizer have dampened his enthusiasm for this project. Here also, however, the academic interest is still alive and unsatisfied. It seemed of general interest, therefore, to summarize briefly the more recent work on this question of nitrogen loss, with particular emphasis on the need for circumspection in evaluating such data.

The question of nitrogen transformations in the soil has been ably reviewed by Grimbert (21), Gage (19), Hulme (27), and others, and the present views of agricultural bacteriologists seem to be adequately stated in the recent texts (12, 25, 29, 34, 38). The nitrogen losses by cropping and by the washing out of soluble salts do not concern this discussion, which is confined to the losses occasioned by free nitrogen production.

The power to reduce nitrate, under favorable conditions, to nitrogen or nitrogen oxides is possessed by relatively few bacteria (29). Most textbooks in discussing the pioneer work of Maassen, imply that 85 of the 109 organisms studied had this power of complete nitrate reduction, whereas the 85 for the most part showed only partial reduction to nitrite or ammonia. Gage (18), in a study of 5300 cultures, also found that 85 per cent of them could reduce nitrate more or less completely. The same author (19) ran complete nitrogen balances on 30 cultures grown in peptone broth with and without the addition of nitrate. When the results falling within the range of experimental error were omitted, the remainder comprised 22 cultures either losing or gaining nitrogen in the peptone alone and 23 losing or gaining in the presence of nitrate. Clearly the nitrate did not favor any marked loss of gaseous nitrogen. These observations are not in accordance with the well-known findings of Stoklasa and Vitek (49) and of Grimbert and Bagros (22). More recently Groenewege (24) has demonstrated the production of nitrous oxide in the fermentation of formates in the presence of nitrate, and Lemoigne and Dopter (31) report losses varying up to 57 per cent after one month and 78 per cent after two months when denitrifying species were grown in legume bouillon to which purine and nitrate had been added; these losses were believed to depend upon autolysis of dead cells in an over-crowded culture. Other less common cases of nitrogen loss are on record and are of great theoretical interest; e.g. the production of nitrous oxide by *B. hyponitrosus* of Mair (33), and the well-known *Thiobacillus denitrificans* of Beijerinck.

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It seems undeniable that certain bacterial types under suitable conditions can occasion nitrogen losses either as the gas or as its oxides. These conditions are usually regarded (29) as the presence of nitrate, the presence of specific microörganisms, excess of readily assimilable organic food, and limited access of oxygen. To these might be added the favorable reaction of the medium which varies with the different organisms (2, 44, 45) and favorable temperature (26). It is also generally conceded (30, 50) that all the necessary conditions rarely occur in practice at any one time, though an improperly handled manure heap may show large losses (14, 15, 37), and there is some evidence of similar losses in flooded soils (2, 30). Errors in interpreting pot cultures or solution cultures in terms of field conditions have frequently been discussed (13, 39, 48) and need not be reviewed here. The interesting work of Prince (41) on the coefficient of variability in soils with nitrogen determinations as criteria is likewise very instructive, and the need for long-continued experiments with statistical treatment of the data is becoming more generally appreciated.

Studies on the chemical nature of the nitrogenous matter in soil (4, 28, 43) and the effects of chemical compounds on denitrification (5, 9, 17, 32, 47) are paving the way for a complete explanation of the phenomenon and of nitrogen loss. The production and spontaneous degradation of ammonium nitrite is an attractive possibility, but essentially without proof. The recent work of Fowler and Kotwal (16) on the stability of NH_4NO_2 solutions (up to 0.15 per cent) even at 50°C. discredits this hypothesis, though the possibility of catalytic decomposition in the soil remains. For example, Hofmann and Buhk (26) have demonstrated the catalytic effect of copper on this reaction; their explanation of the effect has been attacked by Dhar (11), but the facts were not altered.

The equation (27): $2KNO_2 + 3H_2 + 2CO_2 = 2KHCO_3 + 2H_2O + N_2$, which has recently been wandering through our literature in an unbalanced form, is even less probable from the standpoint of free-energy considerations than is the direct oxidation of ammonia to nitrogen and water which has been revived by Mooser (35). Nitrogen loss from amino acid deamination by nitrous acid liberated from nitrites by organic acids has also been attacked by Fowler and Kotwal (16). Apparently this hypothesis, like the ammonium nitrite one, has limited its span of life by being susceptible to experimental verification. At present though, we cannot go much behind the statement that bacteria, under certain inadequately defined conditions, are capable of destroying nitrate with the liberation of nitrogen or its oxides, by a mechanism which is either variable or unknown.

Further evidence of nitrogen losses is forthcoming from the sanitary field.

Five years ago the authors had the opportunity to review (6) the literature on the nitrogen cycle as applied to the aerobic treatment of sewage, and discussed the loss of gaseous nitrogen from (*a*) the reduction of nitrates, (*b*) the oxidation of ammonia, and (*c*) the decomposition of ammonium nitrite. Up to that time a critical review of the literature on the activated sludge process and the reduction of nitrates by sewage sludge, together with the authors' nitrogen balance over a 9-month period, failed to reveal conclusive evidence of nitrogen loss or gain. If such losses occurred, the improbable assumption must be made that simultaneous loss and fixation occurred at substantially the same rate, for in such aeration experiments the gaseous phase is not under quantitative control.

Ardern (2) and his collaborators also found neither loss nor gain in normal tank operation, but with very vigorous nitrate production some loss was indicated. Reddie (42), working with a sewage abnormally high in nitrogen, found losses of 8.6 and 18.6 per cent. In a recent paper, Cavel (10) reports nitrogen losses on aerating activated sludge in the presence of ammonium salts. The one experiment reported in detail, using ammonium sulfate, showed a loss of 42.4 per cent. From the meager details the experimental method seems to be valid, though the concentration of ammonium salt used (2 gm. per liter) is many times greater than any encountered in sewage practice. Although accentuated changes in nitrogen content are desirable to minimize sampling and analytical errors, one is scarcely justified in assuming that metabolic processes follow a constant course even when the normal concentration of food material is increased 50- or 100-fold.

Another form of nitrogen loss is thought to result during the anaerobic digestion of sewage solids. The gases arising from these digestions usually contain appreciable amounts of nitrogen, an observation which is supposed to date back to Angus Smith (1) in 1867. The strictly anaerobic experiments of Popoff (40), however, showed no nitrogen in the gas until he opened and aerated the bottles; the nitrogen content then rose to 53.62 per cent, but after three weeks had again become zero. In the work of Scheringa (46) nitrates and nitrites were intentionally added and the evolved gases were found to contain NO, N_2O, N_2, and NH_3. Groenewege (23) explains the presence of nitrogen in the gases of a sewage tank by assuming the oxidation of ammonia to nitrate in the surface layer and the subsequent diffusion of the nitrate down into the anaerobic regions of the tank. His experimental evidence, although quite extensive, is not very convincing, and it has been pointed out by Buswell and Strickhouser (8) that the nitrogen arising from a digestion tank receiving a steady flow of sewage can be accounted for as dissolved nitrogen swept out of solution by the other gases. Bach and Sierp (3) believed that the intervention of nitrate was unnecessary and that bacterial degradation of nitrogenous matter could result in nitrogen gas production. To decide this point they ran strictly anaerobic laboratory experiments over a period of 268 days. The evolved gases were measured and analyzed and daily samples of liquor were removed by displacement with tap water. The gas analyses are unfortunately given by them only as monthly maxima and minima which the present authors have averaged to obtain the approximate volume of nitrogen evolved. For their most complete experiment from January to August, a total evolution of 1636.1 cc. nitrogen gas can be calculated, and since nitrate and nitrite were never detected in the daily 500-cc. samples, the authors present these data as evidence of nitrogen evolution from purely digestive processes. It is very regrettable that such a carefully conducted experiment cannot be accepted as decisive, for if the dissolved nitrogen in the 500-cc. daily additions of tap water is calculated over the 268 days a total of 1648 cc. of dissolved nitrogen added is found, which is essentially the amount recorded by their accurate analytical control. In some recent, but much less extensive, experiments, the authors (7) have been able to confirm this solubility effect: over a 63-day period, 2860 cc. of gas were collected over 8 liters of water and found to contain 3.7 per cent nitrogen; a calculation shows

$$\begin{aligned} N_2 \text{ in gas} &= 105.8 \text{ cc.} \\ N_2 \text{ in water} &= 98.4 \text{ cc. for average room temperature} \end{aligned}$$

Until these experiments have been repeated with due regard for the solubility and diffusion of atmospheric gases, the balance of evidence must rest in favor of the early findings of Popoff.

Probably an ideal experiment has never yet been performed in science, but a survey of the literature on the question of gaseous nitrogen loss shows at least the need for great caution in interpreting some of the best experimental reports and for more adequate verification of many current hypotheses.

REFERENCES

(1) Aikman, C. M. 1894 Manures and the Principles of Manuring. New York.

(2) Ardern, E., Jepson, C., and Gaunt, P. 1923 Recovery of nitrogen from sewage in the activated sludge process. *Jour. Soc. Chem. Indus.* 42: 230–4T.

(3) Bach, and Sierp, F. 1923 Zur Frage der Stickstoffbildung in Abwasserfaulkammern. *Centbl. Bakt.* (II) 58: 401–11; 59: 1–7.

(4) Bartel, C. 1925 Utilization in the soil of the nitrogen of manure. *Kgl. Landtbruks Akad. Handl. Tid.* 1925; *Rev. Internatl. Renseign. Agr.* 3: 468–469.

(5) Beijerinck, M. W. 1920 Die Chemosynthese bei der Denitrifikation mit Schwefel als Energiequelle. *Koninkl. Akad. v. Wetensch. Amsterdam* 28: 845–856.

(6) Buswell, A. M., and Neave, S. L. 1922 Activated sludge studies, 1920-22. *Ill. State Water Survey Bul.* 18: 68–81.

(7) Buswell, A. M., and Neave, S. L. 1927 The bacterial degradation of sewage grease. *Indus. and Engin. Chem.*, 19: 1012–1014.

(8) Buswell, A. M., and Strickhouser, S. I. 1926 Some observations on sewage tank gases. *Indus. and Engin. Chem.* 18: 407–412.

(9) Caron, H. v. 1912 Untersuchungen über die Physiologie denitrifizierender Bakterien. *Centbl. Bakt.* (II) 33: 62–116.

(10) Cavel, L. 1925 Sur les pertes d'azote dans l'épuration des eaux e'égout par le procédé des boues activées. *Compt. Rend. Acad. Sci.* 181: 1101–1103.

(11) Dhar, N. R. 1921 Einwirkung von Metallen, wie Kupfer und Zink, auf eine wässrige Lösung von Ammonium nitrit. *Ztschr. Anorgan. u. Allg. Chem.* 119: 174–176.

(12) Ellis, D. 1909 Outlines of Bacteriology. London.

(13) Ehrenberg, P. 1909 The nitrogen economy in cultivated soils. *Landw. Ztg.* (Fuhling) 58: 241–246.

(14) Ehrenberg, P., and Reichenbach, E. 1909 Decomposition of manure. *Mitt. Landw. Inst. Breslau* 4: 853–872.

(15) Ferguson, M., and Fred, E. B. 1908 Denitrification—effect of fresh and well-rotted manure on plant growth. *Va. Agr. Exp. Sta. Ann. Rpt.* 1908: 134–149.

(16) Fowler, G. J., and Kotwal, Y. N. 1924 Chemical factors in denitrification. *Jour. Indian Inst. Sci.* 7: 29–37.

(17) Fred, E. B. 1911–12 Eine physiologische Studie über die nitratreduzierenden Bakterien. *Centbl. Bakt.* (II) 32: 421–449.

(18) Gage, S. DeM. 1901 33rd Annual Report. *Mass. State Bd. of Health*, 1901: 401, 419.

(19) Gage, S. DeM. 1905 Contribution to the biochemistry of sewage purification; the bacteriolysis of peptones and nitrates. *Jour. Amer. Chem. Soc.* 27: 327–63.

(20) Gerretsen, F. C. 1921 Eine Untersuchung auf Nitrifikation und Denitrifikation in tropischen Böden. *Arch. Suikerindus. Nederland. Indië, Verslag.* 29: 1397–1532.

(21) Grimbert, L. 1904 Les bactéries dénitrifiantes et le méchanisme de la dénitrification. *Bul. Inst. Pasteur* 2: 937–947.

(22) Grimbert, L., and Bagros, M. 1909 Sur le méchanisme de la dénitrification chez les bactéries dénitrifiantes indirectes. *Compt. Rend. Soc. Biol.* 66: 760–763; *Jour. Pharm. et Chim.* (6) 30: 5–10.

(23) Groenewege, J. 1920 Bakteriologische Onderzuekingen over Biologische Reiniging. *Meded. Burgerl. Geneesk. Dienst (Dutch Indies)* D.1: 66–125.

(24) Groenewege, J. 1921 Denitrification with formates and the influence of the cation on this process. *Meded. Alg. Proefsta. Landb.* 7.

(25) Hiss-Zinsser 1922 Text Book of Bacteriology, 5th ed. New York.

(26) Hofmann, K. A., and Buhk, G. 1920 Reaktionen von Salzen der salpetrigen Säure mit schwachen Basen. *Ber. Deut. Chem. Gesell.* 53: 2165–71.

(27) Hulme, W. 1914 The mechanism of denitrification. *Jour. Chem. Soc.* 105 (1): 623–32.

(28) Jodidi, S. I. 1911 The chemical nature of the organic nitrogen in the soil. *Jour. Amer. Chem. Soc.* 33: 1226–1241.

(29) Jordan, E. O. 1922 General Bacteriology, 7th ed. Philadelphia.

(30) Lemmermann, O., et al. Verlauf der Denitrifikation in Böden bei verschiedenem Wassergehalt. *Centbl. Bakt.* (II) 41 (1914): 608–25; also *Landw. Jahrb.* 38 (1909): 319; 41 (1912): 163–216.

(31) Lemoigne, M., and Dopter, P. L. 1926 Sur les pertes d'azote provoquées les bactéries du sol en cultures pures. *Compt. Rend. Acad. Sci.* 183: 160–162.

(32) Lumia, C. 1923 Accelerating action of amides on the denitrification processes in the soil. *Ann. Chim. Appl.* 13: 155–160.

(33) Mair, W. 1908 On the rôle of bacteria in the biological methods of sewage purification with special reference to the process of denitrification. *Jour. Hyg.* 8: 609–653.

(34) Marshall, C. E. 1921 Microbiology, 3rd ed. Philadelphia.

(35) Mooser, W. 1911 Biologisch-chemische Vorgänge im Erdboden. Beiträge zur Stickstoff-frage. *Landw. Vers. Sta.* 75: 53–106.

(36) Nechaev, N. B. 1923 An investigation of the process of denitrification in the Neva River (Russia). *Isvestia R. G. I.* 12: 1–18; abs. in *Chem Abs.* 19 (1925): 1921.

(37) Niklewski, B. 1923 Diminution du taux de l'azote dans le fumier sous l'influence des bactéries nitrifiantes. *Rocz. Nauk Rolnicz.* 9: 193–210; *Bul. Soc. Chim. Biol.* 5: 491–500.

(38) Percival, J. 1920 Agricultural Bacteriology, 2nd ed. London.

(39) Pfeiffer, T., et al. 1909 The nitrogen economy in cultivated soils. *Mitt. Landw. Inst. Breslau* 4: 715–851.

(40) Popoff, L. 1875 Ueber die Sumpfgasgährung. *Pflüger's Arch. Physiol.* 10: 113–146.

(41) Prince, A. L. 1923 Variability of nitrates and total nitrogen in soils. *Soil Sci.* 15: 395–405.

(42) Reddie, J. A. Quoted in *Soc. Chem. Indus. Ann. Rpt.* 1926: 574–575.

(43) Russell, J. 1923 The Micro-organic Population of the Soil. London.

(44) Sacharowa, T. M. 1923 *Arb. Wiss. Inst. Düngemittel* No. 15, 1–22.

(45) Sacharowa, T. M. 1925 Die Abhängigkeit der Denitrifikationsgeschwindigkeit von der Reaktion des Mediums. *Centbl. Bakt.* (II), 65: 15–35.

(46) Scheringa, K. 1920 Denitrification by bacteria and its relation to water analysis. *Rpt. Cent. Lab., Pub. Health Serv. (Netherlands)* 1920: 165–171.

(47) Scheringa, K. 1920 Ist die chemische Denitrifikation von Bedeutung für Akerbau und Wasseruntersuchung? *Pharm. Weekbl.* 57: 1481–1483.

(48) Stevens, F. L., Withers, W. A., et al. 1909 Studies in soil bacteriology. *Centbl. Bakt.* (II) 23: 355–73; 776–785.

(49) Stoklasa, J., and Vitek, E. 1906 Über den Einfluss verschiedener Kohlenhydrate und organischer Säuren auf die Denitrifikationsprozess. *Ztschr. Zuckerindus. Böhmen.* 31: 67–119.

(50) Voorhees, E. B., and Lipman, J. G. 1909 Experiments on the utilization of nitrogen in fertilizer materials. *N. J. Agr. Exp. Sta. Bul.* 221: 3–52.

ELECTRODIALYSIS OF SOILS AND THE MATTSON CELL

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During the last few years, interest in exchange of bases by soils has been on the increase. This has been due, in a great measure, to the experiments of Hissink (4, 5) in Holland and to the translation from the Russian (3) of the reports of Gedroiz. The revival of interest has added not only to the number of workers, but also to the variety of ways in which the subject can be taken up. Among the many methods, the application of electrodialysis by Sante Mattson (9) introduces a very promising line of attack on the base exchange problem.

Electrodialysis was used by Cameron and Bell (1) for the removal of bases and of the hydrolyzed products of minerals; it was also used by König and his co-workers (6, 7, 8) on a more extensive scale. Last year, Mattson made a preliminary study of the exchangeable bases of the soil removed by N ammonium chloride and 0.05 N hydrochloric acid compared with those obtained by electrodialysis, both from the soil itself and from the colloidal fraction. He found that the quantity removable by electrodialysis was fairly definite, and agreed with the yields from the treatment with the ammonium chloride and the acid.

Mattson designed the three-chambered cell shown in figure 1. The middle compartment enclosed the soil between parchment paper—the paper being braced by a glass lattice. The cell was made of soft rubber of U-shape (B_1, C, B_2) except for the ends, which were of hard rubber (A_1, A_2). The whole was fastened together by a number of bolts. Electrodes (E_1, E_2) were made of platinum wire gauze and were large enough to extend to the sides and bottom of the chambers. Outlets (O_1, O_2) were provided to draw off the solution in the outer compartments. The central chamber for the soil being narrow, the resistance to a 220-volt direct current (with a 50- or 25-watt lamp in series) did not cause the temperature to rise above 50°C. The experiments here reported are concerned with the technique used in the handling of this type of cell.

EXPERIMENTAL

A cell similar to the one described was prepared and found to work very satisfactorily, although the hard rubber outer ends warped somewhat; apparently these also could be made, with advantage, of thin soft rubber, possibly backed with a metal sheet. Parchment paper, previously dialyzed to remove sulfates, as described by Mattson, was used for the inner chamber. The cost of the cell, excluding the platinum, is not excessive. Two cells can be cut from about $20 worth of rubber. The gauze electrodes, 5 by 3 inches, were made from 52-mesh, 0.004-inch wire weighing about 0.6 gm. per square inch: the two cost $60. A considerable part of the expense can be saved as

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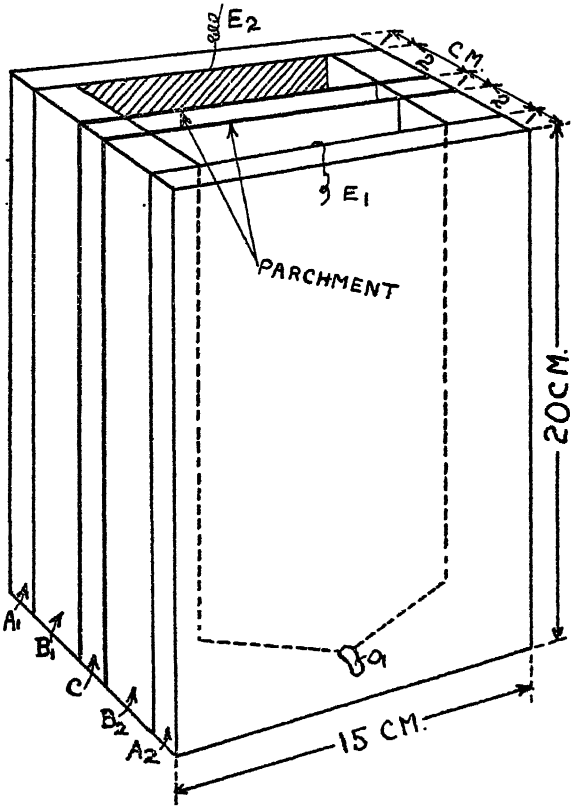

FIG. 1. THE MATTSON CELL FOR ELECTRODIALYSIS OF SOILS

is indicated in the following discussion on electrodes (p. 294). It was found advisable to seal a small glass rod to the top and bottom of the gauze to prevent tearing. Direct current at the start of the work had a voltage of 110 to 120 and was used at first with a 40-watt lamp in the circuit, but afterwards without any resistance. The current was allowed to run continuously: the ammeter reading did not go above 1.00 with 100 gm. of any except one peat soil.

Rate of extraction

In Mattson's experiments the rate at which the electrodialysis proceeded was determined by titration of the dialysates with phenolphthalein as the indicator. In this way he obtained from a Sharkey clay soil 2.655 milliequivalents of bases in the first hour; the amount gradually dropped, until at the twenty-second hour only 0.090 milliequivalents were found. At the end of 42 hours titratable bases were less than 0.01 milliequivalents per hour, and this proved a fairly definite end point. On the acid side, the first hour produced 0.860 milliequivalents and at the end of 22 hours, 0.060. The values fell very quickly after the first 12 hours. In a loam soil the figures fell after the third hour and very small total amounts were obtained.

In the authors' early experiments on a Carrington Loam they obtained results very similar to Mattson's on the Sharkey clay. One run was made on 100 gm. of soil, the dialysate being withdrawn every hour for the first 56 hours, in which the temperature maximum was 44°C. At the end of 19 hours 18.15 milliequivalents had been removed on the basic side and 1.86 on the acid. From that time to the end of the 56 hours the amounts of the bases were not measurable by phenolphthalein, but continued on the acid side at about 0.04 milliequivalents per hour. The extraction was continued for 6 days, the dialysate being withdrawn at intervals of 6 to 12 hours. The last change (12 hours) produced 0.19 milliequivalents on the basic side and 0.1 on the acid. The total amounts obtained were 20.8 and 4.2.

When the time of changing the dialysate was altered to 6- and 12-hour intervals, the temperature rose to 50°C. At the end of 24 hours (3 changes) 18.6 milliequivalents of bases had been removed, followed by 1.1 in the next 6 hours—a further 35 hours brought only 0.4 milliequivalents. The constant changing was thus shown to be unnecessary.

Voltage change

In order to make the method of general application it was necessary to use some way of converting A.C. current to D.C., as direct current is not available in many laboratories. A small 7.5 to 15-volt rectifier on the 110 A.C. was tried, but this gave too small a current. At the end of seven days there was still a considerable amount of the bases not withdrawn. This was therefore abandoned.

A more promising lead was found by connecting an A.C. motor to run a D.C. motor reversed—both of ⅛ H.P. The voltage obtained by this method was frequently above 50 and the temperature in the cell rose to 44°C. The dialysis proceeded almost as fast as with the direct 110 volts. A ¼ H.P. should prove suitable for this size of cell.

Cooling

Although no attempt was made to obtain thermostatic control, a modification of the Mattson cell was introduced by substituting for the glass rods on each side of the parchment paper, two thin glass tubes bent into the shape of a grid, to form a cooling system. A further grid was placed in the center compartment so that the system could be kept at any desired temperature by running water through the tubes. There is ample room in the center for 100 gm. of soil as well as for the cooling tubes. An experiment in which air was bubbled through the soil, considerably slowed the electrodialysis, as was expected.

Electrodes

Several experiments were made to cut down the cost of the platinum gauze electrodes. The substitution of a very small piece of sheet platinum for one of the electrodes, while keeping the gauze in the opposite compartment, increased the time needed for the dialysis. An electrode was made of no. 22 platinum wire, trident shaped, with a glass rod at the bottom to hold it stiffly. This proved very successful. Such a wire electrode, costing not much more than one-third the price of the gauze, can be substituted for one electrode without noticeably delaying the dialysis. The wire has the added advantage that the electrode is much more easily washed free from the precipitate which forms in the later stages of the process. Wires in both compartments in place of the gauze, slowed the action considerably.

In many cases it would be possible to use cheaper metals on the basic side. Copper gauze as the cathode and the platinum three wire as the anode gave the fastest dialysis—possibly because the gauze was rather larger than the platinum gauze.

Changing the Dialysate

As indicated previously, in the early experiments the dialysate was changed every hour, but this was soon found to be unnecessary in order to get the quickest dialysis. Changes at 3, 10, 24, and 48 hours are sufficient to bring out practically all the dialysate. No change during the first 27 hours slowed down the speed materially. The final method adopted consisted of two intervals, the first of 3 hours and the second of 6, followed by 12-hour changes until the extraction was complete. As a rule, after the first 24 hours, little base or acid was removed from the soil, although the total time needed varied with the type of soil: a peat took very much longer for complete dialysis.

Mattson found that he had a very good check for the exchangeable bases from *N* ammonium chloride or from 0.05 *N* hydrochloric acid, when he stopped the electrodialysis at the end point of phenolphthalein. The authors found that when the dialysis was continued beyond this point, bases continued to be produced, shown by methyl red, with usually a rather heavy precipitate, which dissolved on acidifying. Frequently a very indefinite end point was secured with the methyl red. From what part of the soil this precipitate comes or what is its nature has not yet been determined.

That this type of cell has application along other lines is indicated by the fact that in these laboratories it has been found that there is a distinct difference in rate of dialysis of apple peelings from healthy and from diseased apples.

SUMMARY

1. The Mattson Cell for electrodialysis of soils has proved very satisfactory.
2. A method for obtaining a direct current suitable for the cell is described.
3. Variations in the electrodes in order to use less platinum are suggested.
4. A cooling system has been introduced into the cell, and the rate of extraction, under various conditions, investigated.

REFERENCES

(1) CAMERON, F. K., AND BELL, J. M. 1905 The mineral constituents of the soil solution. U. S. Dept. Agr. Bur. Soils Bul. 30.

(2) GEDROIZ, K. K. 1923 The hydrochloric acid method for determining in the soil the cations present in an absorbed condition. *Soil Sci.* 16: 473–474.

(3) GEDROIZ, K. K. 1912–1924. Papers in *Zhur. Opit. Agron.* (English translation by S. A. Waksman. Mimeographed copies from Dr. Schofield, U. S. Dept. Agr.)

(4) HISSINK, D. J. 1923 Method for estimating adsorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.

(5) HISSINK, D. J. 1924 Base exchange in soils. *Trans. Faraday Soc.* 1924: 551–566.

(6) KÖNIG, J., HASENBÄUMER J., AND HASSLER C. 1911 Bestimmung der Kolloide im Ackerboden. *Landw. Vers. Sta.* 75: 377–441.

(7) KÖNIG, J., HASENBÄUMER, J., AND GLENK K. 1913 Über die Anwendung der Dialyse und die Bestimmung der Oxydationskraft für die Beurteilung des Bodens. *Landw. Vers. Sta.* 79–80, 491–534.

(8) KÖNIG, J., HASENBÄUMER, J., AND KUPPE, K. 1923 Beziehungen zwischen den im Boden vorhandenen und den von Roggen und Futterrüben aufgenommenen leichtlöslichen Nährstoffen. *Landw. Jahrb.* 59: 65–96.

(9) MATTSON, S. 1926 Electrodialysis of the colloidal soil material and the exchangeable bases. *Jour. Agr. Res.* 33: 553–568.

BOOK REVIEW

Soil Mineralogy. By Frederick A. Burt, Associate Professor of Geology, A. and M. College of Texas. D. Van Nostrand and Co. Inc., New York, 1927. vii, pp. 82, illus. 6. Price $1.50.

The author presents a very concise treatise of mineralogy from the point of view of the occurrence, estimation and importance of minerals in agricultural soils. Purposely there is an "omission of all minerals not directly concerned with soil formation or used as mineral fertilizer." The book is divided into three parts followed by tables of occurrence, weathering, and physical properties of minerals. Part 1 treats of general principles, giving many definitions and methods of study, and considerable discussion of the elements and of rock weathering. Part 2 very briefly presents a key for the identification of minerals. Part 3 occupies nearly half of the treatise in descriptions and discussions of each of the mineral species.

The subject material is presented in very lucid style and appears to be well adapted for the use of students beginning soil mineralogy. A somewhat more comprehensive treatment would undoubtedly find wider application.

R. L. Starkey.

SOME OF THE FACTORS WHICH INFLUENCE THE COMPOSITION OF CABBAGE AND THEIR RELATION TO THE QUALITY OF SAUERKRAUT[1]

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INTRODUCTION

A knowledge of the composition of the raw product is of fundamental importance in the study of any fermentation process. Variations in the composition of the cabbage may influence the type of fermentation as well as the end products of fermentation in the making of sauerkraut. In a previous publication (11) attention was called to the variation in sugar, total nitrogen, and calcium in cabbage from various parts of the United States, and also to the relation between these constituents and the quality of the sauerkraut. It is the purpose of this paper to show the effect of various factors on the composition of cabbage, such as the type of soil on which it is grown, the age at which it is harvested, the variety used, and the time and temperature at which it is stored, and to determine the effect which these factors have on the quality of sauerkraut made from the cabbage.

EXPERIMENTAL

The investigations reported extended over a period of two years, 1925 and 1926. The nature of the work was essentially the same for both years but the data are recorded separately. As far as possible, however, general conclusions are drawn from the results of the two seasons.

Variety of cabbage. The cabbage used in 1925 was of the All Seasons variety, excepting that shipped from Racine, Wisconsin, which was either Glory, All Head, or All Seasons. In 1926, Early Copenhagen and All Seasons were used.

Soil. In 1925 the cabbage raised on the university farm was grown on two plots, one of which was Miami silt loam and the other alkaline marsh. In 1926 the same marsh plot, but a different plot of Miami silt loam was used. The cabbage sent from Racine during the 1925 season was grown on Clyde silt loam.

Maturity. The cabbage was set out in the early part of June in both years; that on the marsh about a week before that on the Miami silt loam. The heads grown on the University plots were cut at three different times during the season to determine what effect stage of maturity has on the composition of the cabbage. Unfortunately the last cuttings of both years were made after the cabbage was rather badly frozen.

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[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] The authors are indebted to R. J. Allgeier for assistance in some of the analytical work.

Storage. In order to study the effect of storage on the composition of the cabbage tissue, heads were placed in piles, and allowed to remain for various lengths of time at a temperature of 70°F. or higher. In 1926, however, during the last part of the season it was necessary to cut the cabbage and pile it to protect it from freezing. At this time the cabbage was piled outdoors, covered with canvas, and kept under very cold conditions for 2 to 4 weeks.

Sauerkraut. All of the cabbage, the analyses of which appear in the subsequent tables, was made into sauerkraut. The usual factory methods of coring and shredding the cabbage were employed. Two and one-half per cent of salt was added to the cabbage, which was then packed into 10-gallon stone jars or 45-gallon barrels. Heavy weights were placed on top of the shredded cabbage in the jars or barrels. The rate of fermentation was followed by titrating the juice at frequent intervals.

ANALYTICAL METHODS

Moisture. Twenty grams of material was dried for 24 hours in an oven at 105°C. The loss in weight was calculated as percentage of moisture in the original tissue.

Total nitrogen. For the Kjeldahl nitrogen determinations, 10 gm. of finely chopped cabbage was used.

Water-soluble constituents. Four hundred grams of finely cut cabbage was ground in a mortar with a little ether and well washed quartz sand, until all of the cells were ruptured. The mass was then strained through cheese cloth, filtered through paper pulp by means of suction, washed, and finally made up to 2 liters. This solution was analyzed for *total nitrogen* by the Kjeldahl method, for *amino nitrogen* by the Van Slyke (13) procedure, and for *reducing sugars,* before and after hydrolysis of the clarified solution, by the procedure of Shaffer and Hartmann (12). *Sucrose* was calculated from the two determinations of reducing sugars. *Calcium* and *phosphorus* were determined in both the water solutions and the original tissue. The McCrudden (8, 9,) method was used for calcium, and the volumetric method of the Association of Official Agricultural Chemists (2), for phosphorus.

About 1 kgm. of the original material was preserved for various purposes. This was done by heating the tissue at 70°C. for ten minutes, and then drying at 40°C. for several days.

DISCUSSION OF RESULTS

Tables 1 and 3 contain a description of the cabbage used in 1925 and 1926 and indicate the relation of various factors to the quality of sauerkraut. The

TABLE 1

Relation of variety of cabbage, soil type, storage, maturity, and freezing to quality of sauerkraut (1925)

SAMPLE NUMBER	SOIL TYPE	VARIETY	DATE OF CUTTING	SAUERKRAUT				
				Titratable acid as lactic	Taste	Color	Texture	General grading
				per cent				
347	Clyde silt loam	Glory	September 16	1.647	Excellent	Good	Good	Good
348		All Head stored 7 days	September 16	0.828	Bad	Pink	Bad	Very poor
351		All Seasons	October 15	1.386	Fair	Good	Tough	Fair
353		351 stored 15 days	October 15	1.467	Bad	Good	Tough	Poor
349	Alkaline marsh	All Seasons	September 29	1.404	Fair	Dark	Soft	Fair
352		All Seasons	October 23	1.197	Fair	Good	Fair	Fair
355		All Seasons frozen	November 11	1.440	Poor	Good	Soft	Very poor
350	Miami silt loam	All Seasons	October 6	1.449	Good	Dark	Tough	Fair plus
354		All Seasons	October 28	1.620	Good	Yellow	Fair	Good
356		All Seasons frozen	November 11	1.539	Good	Excellent	Soft	Good
357	*	All Seasons	November 5	1.755	Good	Very good	Fair	Good
358	*	All Seasons frozen	November 5	1.917	Poor	Poor	Poor	Poor

* Factory experiment, soil type unknown.

cabbage grown on the upland soil, as a rule, made better sauerkraut than that from the marsh. This is largely because the heads from the former grew more slowly and were firmer than those from the marsh. A firm head usually makes sauerkraut of better cut than one which is loose and coarse. Late cabbage, in general, makes better sauerkraut than early cabbage. The latter is often green, and produces a yellow kraut. Storage of cabbage during cool weather has apparently little effect on the quality of the sauerkraut, but cabbage which is stored during August and September becomes very warm and is likely to become covered with undesirable microörganisms. This may lead to an abnormal and undesirable fermentation in the vats. If the freezing is not too severe, it does not seem to impair the kraut-making value of the cabbage. If the freezing is so severe that the tissue is mechanically injured, a soft, slippery kraut is the result. Variations in the composition of cabbage grown in 1925 are recorded in table 2.

TABLE 2

Relation of variety of cabbage, soil type, storage, maturity, and freezing to composition of cabbage (1925)

(Calculations based on the fresh cabbage)

SAMPLE NUMBER	MOISTURE	TOTAL NITROGEN	WATER SOLUBLE NITROGEN	AMINO NITROGEN	REDUCING SUGAR AS GLUCOSE	SUCROSE	CALCIUM		PHOSPHORUS	
							Total	Water-soluble	Total	Water soluble
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
347	93.2	0.18	0.12	0.07	2.29	0.54	0.045	0.030		
348	93.3	0.13	0.09	0.07	2.35	0.68	0.038	0.025		
351	93.2	0.20	0.11	0.08	2.60	0.53	0.043	0.028	0.028	0.020
353	92.9	0.21	0.11	0.05	2.57	0.62	0.046	0.023	0.028	0.014
349	93.8	0.24	0.16	0.12	2.44	0.44	0.050	0.030	0.025	0.016
352	93.8	0.21	0.14	0.09	2.06	0.36	0.051	0.029	0.024	0.015
355	93.9	0.23	0.13	0.08	1.35	0.15	0.053	0.022	0.027	0.011
350	93.1	0.21	0.14	0.08	2.79	0.21	0.042	0.028	0.030	0.024
354	93.9	0.21	0.12	0.05	2.64	0.18	0.043	0.025	0.036	0.022
356	93.8	0.24	0.12	0.09	1.26	0.17	0.047	0.029	0.033	0.013
357	93.5	0.21	0.14	0.12	2.28	0.27	0.046	0.029	0.027	0.017
358	93.8	0.21	0.13	0.11	1.71	0.05	0.045	0.028	0.023	0.018
Aver.	93.6	0.20	0.13	0.08	2.10	0.36	0.046	0.028	0.028	0.017

Moisture. The amount of moisture varies but little in the different samples. The stored cabbage (no. 353) contained the least moisture of all the samples and showed a slight loss as compared with the freshly harvested sample (no. 351).

Nitrogen. Approximately three-fifths of the nitrogen exists in the form of soluble compounds and of these about 60 per cent is in the form of amino nitrogen. This percentage is approximately the same as that found in the hydrolysis products of a protein and suggests that the water-soluble forms of nitrogen are simple structures.

TABLE 3

Relation between variety of cabbage, soil type, storage, maturity and freezing to quality of sauerkraut (1926)

SAMPLE NUMBER	SOIL TYPE	VARIETY	DATE OF CUTTING	SAUERKRAUT				
				Titratable acid as lactic	Taste	Color	Texture	General grading
				per cent				
402	Alkaline marsh	All Seasons	September 13	1.359	Fair	Fair	Fair	Fair
403		All Seasons stored 11 days	September 13	0.972	Good	Mottled	Good	Fair plus
404		All Seasons	October 1	1.008	Fair	Good	Tough	Fair plus
407		All Seasons frozen	October 25	1.422	Good	Good	Good	Very good
401	Miami silt loam	Early Copen.	August 23	1.602	Good	Good	Tough	Very good
405		All Seasons	October 12	1.332	Good	Good	Good	Very good
406		All Seasons	October 20	2.043	Bad	Good	Soapy	Poor
408		All Seasons	October 25					
409		All Seasons	October 30	1.692	Very good	Very good	Very good	Very good
410		All Seasons frozen	November 19	1.791	Very good	Very good	Very good	Very good
411		All Seasons	Badly frozen November 27					
F26-1	*	Early Copen.	August 27	1.60	Good	Fair	Good	Good
F26-2	*	Early Copen. stored 7 days.	August 27	1.82	Good	Good	Good	Good
F26-3	*	All Seasons outside of pile	October 15					
F26-4	*	All Seasons center of pile	October 15					

*Factory experim ype unknown.

The effect of an abundance of nitrogen in the soil is reflected in the marsh cabbage. This is richer in all forms of nitrogen than the cabbage from the two upland soils. The difference is especially marked in the water-soluble and amino nitrogen. The marsh samples contained from 15 to 35 per cent more of these constituents than the upland samples.

As would be expected, the proportion of highly organized nitrogen increases, and the percentage of simpler forms of nitrogen decreases with maturity. The change is most clearly shown in the amino nitrogen which decreased from 50 per cent of the total in sample 349 to 43 per cent in sample 352, and from 38 per cent in sample 350 to 24 per cent in sample 354.

TABLE 4

Relation between variety of cabbage, soil type, storage, maturity, freezing, and composition of cabbage (1926)

(Calculations based on the fresh cabbage)

SAMPLE NUMBER	MOISTURE	TOTAL NITROGEN	WATER SOLUBLE NITROGEN	AMINO NITROGEN	REDUCING SUGAR AS GLUCOSE	SUCROSE	CALCIUM	PHOSPHORUS	
							Total	Total	Water-soluble
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
402	93.7	0.26	0.13	0.07	2.39	0.22	0.056	0.020	0.015
403	93.2	0.23	0.12	0.08	2.34	0.45	0.058	0.020	0.007
404	94.0	0.28	0.12	0.09	1.85	0.33	0.072	0.024	0.008
407	94.6	0.24	0.14	0.08	1.60	0.05	0.062	0.017	0.011
401	92.5	0.30	0.14	0.07	2.38	0.0			
405	92.9	0.23	0.10	0.07	2.45	0.28	0.054	0.033	0.012
406	93.2	0.21	0.11	0.08	3.25	0.27	0.051	0.028	0.012
408	92.0	0.23	0.12	0.08	2.50	0.16	0.049	0.035	0.010
409	92.2	0.22	0.10	0.07	2.43	0.15	0.051	0.034	0.017
410	92.7	0.25	0.13	0.07	2.43	0.54	0.049	0.031	0.015
411	91.9	0.24	0.13	0.07	2.60	0.78	0.053	0.034	0.011
F26-1	94.3	0.18	0.09	0.06	2.02	0.07	0.055	0.023	0.013
F26-2	93.5	0.18	0.10	0.09	1.90	0.34	0.062	0.023	0.013
F26-3	92.3	0.23	0.11	0.06	2.66	0.72	0.072	0.025	0.012
F26-4	90.7	0.24	0.12	0.08	2.59	0.80	0.085	0.035	0.017
Aver.	92.9	0.24	0.12	0.08	2.37	0.29	0.059	0.027	0.012

Sugar. The total sugar consists on the average of about 85 per cent reducing sugars and 15 per cent sucrose. There is from 10 to 20 per cent more sugar in the samples grown on the loam soils than in those grown on the marsh. A similar inverse relationship between nitrogen and carbohydrate has been reported for the tomato by Kraus and Kraybill (6). Storage and maturity both cause a moderate decrease in reducing sugar. Peterson, Fred, and Viljoen (11) observed a similar response to maturity. They found that the sugar was 7.4 per cent lower in late-maturing heads than in early-maturing ones. The most striking change in sugar is brought about by freezing in the field (nos. 355, 356, and 358). As a result of this treatment about fifty per cent of the reducing sugars disappeared. Apparently they were transformed into some other carbohydrate, since the moisture content remained unchanged and the kraut made from this cabbage contained as great a quantity of fermentation products as that made from normal material.

Calcium and phosphorus. It appears that the total calcium is higher in the marsh cabbage than in the upland-grown material. In both cases the amount of calcium increased with maturity. The amounts of water-soluble calcium and phosphorus decreased with maturity and storage.

Variations in the composition of cabbage grown in 1926 are shown in table 4. About the same differences in moisture are brought out in this table as were found in the data of the previous year.

Nitrogen. The same general relations hold for the 1926 samples as existed in the 1925 samples. The marsh samples are higher than the loam samples in total, water-soluble, and amino nitrogen. More of the total nitrogen was present as insoluble and less as water-soluble and amino nitrogen in the 1926 samples than in the 1925 samples. Perhaps the cabbage in 1926 was more matured than that in 1925. In both sets of samples the ratios of water-soluble and the amino nitrogen to total nitrogen decreased toward the end of the growing season. At the maximum, the water-soluble nitrogen comprises 52 per cent of the total, and at the minimum, about 43 per cent. The maximum and minimum figures for amino nitrogen are 38 and 32 per cent respectively. The distribution of the forms of nitrogen is about the same in the marsh and the loam samples.

Sugar. Reducing sugars are higher in cabbage from the upland soil than from the marsh. Maturity, storage, and freezing in the field (no. 407) all lower the amount of this constituent, as was found to be true from the results obtained in 1925. Appleman and Miller (1), working with potato tubers, found that reducing sugars and sucrose are lowered by both maturity and storage. A similar response to storage was observed by Pack (10) in working with sugar beets, by Bigelow and Gore (4) in studying the peach, and by Voelcker (14) in early experiments with Swedish turnips. The losses in sugar noted by these investigators were believed to be due entirely to respiration.

Apparently when the cabbage is cut and stored for a period of 2 to 4 weeks at freezing temperatures (nos. 410 and 411) there is no reduction in the sugar content. Freezing produces this effect on cabbage only before it is harvested (nos. 355, 356, 358, 407).

Calcium and phosphorus. It is evident that the calcium is much higher in marsh cabbage than in that grown on the upland soil. The water-soluble phosphorus decreases with maturity and storage. These results are in agreement with those obtained in 1925. It has been observed in previous work of this kind that the amount of such a constituent as calcium in plant material is largely dependent upon the quantity of this element available in the soil on which the plants are grown. Variations in the mineral content of plant material, with respect to soil type and the fertilizer used on the soil, have been reported by Beal (3), Wheeler and Hartwell (14), Forbes, Whittier, and Collison (5), and Hoagland (6). Analyses show that the alkaline marsh soils of Wisconsin contain much more calcium than the Miami silt loams.

There are several clear-cut environmental factors that effect the composition of cabbage and that can be summarized for the two years as follows:

Cabbage grown on the alkaline marsh contains more total, water-soluble, and amino nitrogen, as well as sucrose and calcium, than that grown on the upland type of soil. However, the former contains less reducing sugars.

Water-soluble nitrogen, amino nitrogen, reducing sugars, sucrose, and water-soluble calcium and phosphorus decrease with maturity, whereas the amount of total calcium is present in somewhat larger quantities in mature heads. The simpler forms of nitrogen compounds and carbohydrates disappear with maturity. They are undoubtedly changed into more complex forms. This also appears to be true of the water-soluble calcium and phosphorus.

Storage appears to lower the amounts of moisture, reducing sugars, and water-soluble calcium and phosphorus in the tissue. Sucrose apparently increases with storage. Freezing the cabbage heads before they are cut causes one very marked effect, namely, that of lowering the reducing sugars and sucrose. The amount of soluble nitrogen is increased by this treatment.

The variety of cabbage apparently has no very definite effect upon the composition of the tissue.

In the formation of sauerkraut, the salts, the nitrogen compounds, and the carbohydrates all play an important rôle. It is probable, however, that in any normal cabbage there is a sufficient amount of all these constituents to sustain a normal fermentation. There seems to be no direct correlation between the chemical composition of the cabbage and its kraut-making qualities. The factors involved in the making of sauerkraut are many and complex. Besides a good quality of cabbage the proper type of fermentation must take place. It is probable that the latter is fully as important, if not more so, than the former.

SUMMARY

Sauerkraut made from late cabbage is of better quality than that made from early cabbage. Cabbage grown on upland soil usually makes better sauerkraut than that grown on marsh soil. Storage at low temperatures and slight freezing of cabbage have little, if any, effect on the quality of sauerkraut. If the cabbage has been severely frozen, or stored at high temperatures, the sauerkraut is likely to be of poor quality. The best sauerkraut is obtained from cabbage which forms firm compact heads. The leaves in the head should be of fine texture so as to give long, slender threads when shredded.

The inorganic constituents, the nitrogen compounds, and the carbohydrates are influenced by the type of soil. A marsh soil produces a cabbage higher in nitrogen and lower in sugar than does an upland soil.

About one-half of the nitrogen compounds in cabbage are soluble in water. These compounds appear to be simple structures, as approximately 60 per cent of their nitrogen is present in free amino groups.

The sugar in cabbage consists of about 85 per cent reducing sugar and 15 per cent sucrose.

The simple constituents of the growing cabbage tissue are transformed into more complex forms as the head matures. Storage reverses these processes and may cause a loss of sugar by respiration. Freezing before cutting apparently converts the sugar into other forms of carbohydrate.

REFERENCES

(1) Appleman, C. O., and Miller, E. V. 1926 A chemical and physiological study of maturity in potatoes. *Jour. Agr. Res.* 33: 569–577.

(2) Association of Official Agricultural Chemists 1925 Official and Tentative Methods of Analysis Compiled by the Committee on Editing Methods of Analysis, 2nd ed. Washington, D. C.

(3) Beal, W. H. 1889 Compilations of the analysis of fodder articles, fruits, sugar-producing plants, dairy products, etc. Mass. Agr. Exp. Sta. Ann. Rpt. 1889: 291–313.

(4) Bigelow, W. D., and Gore, H. C. 1905 Studies on peaches. *U. S. Dept. Agr. Bur. Chem. Bul.* 97: 22–32.

(5) Forbes, E. B., Whittier, A. C., and Collison, R. C. 1910 The mineral nutrients in blue grass. *Ohio Agr. Exp. Sta. Bul.* 222: 39–53.

(6) Kraus, E. J., and Kraybill, H. R. 1918 Vegetation and reproduction with special reference to the tomato. *Ore. Agr. Exp. Sta. Bul.* 149.

(7) Hoagland, D. R. 1919 Relation of nutrient solution to composition and reaction of cell sap of barley. *Bot. Gaz.* 68: 297–304.

(8) McCrudden, F. H. 1910 The quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine and feces. *Jour. Biol. Chem.* 7: 83–100.

(9) McCrudden, F. H. 1911 The determination of calcium in the presence of magnesium and phosphates: The determination of calcium in urine. *Jour. Biol. Chem.* 10: 187–199.

(10) Pack, D. A. 1926 The effect of moisture on the loss of sugar from sugar beets in storage. *Jour. Agr. Res.* 32: 1143–1152.

(11) Peterson, W. H., Fred, E. B., and Viljoen, J. A. 1925 Variations in the chemical composition of cabbage and sauerkraut. *Canner* 61 (4): 19–21.

(12) Shaffer, P. A., and Hartmann, A. F. 1920 The iodometric determination of copper and its use in sugar analysis. *Jour. Biol. Chem.* 45: 349–390.

(13) Van Slyke, D. D. 1912 The quantitative determination of aliphatic amino groups. *Jour. Biol. Chem.* 12: 275–284.

(14) Voelcker, A. 1877 On the composition and nutritive properties of swedes, and experiments on the keeping qualities of roots. *Jour. Roy. Agr. Soc.* (Ser. 2) 13 (Pt. 1): 157–177.

(15) Wheeler, H. J., and Hartwell, B. L. 1906 Concerning the functions of sodium salts. *R. I. Agr. Exp. Sta. Ann. Rpt.* 1906: 186–316.

DECOMPOSITION STUDIES OF ALFALFA AND SWEET CLOVER ROOTS AND STRAW

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Various kinds of organic materials are incorporated with soils. The rate at which they decompose is often considered a measure of their fertility value. Alfalfa and sweet clover are regarded to be among the most valuable ordinary field crops when used as green manures. The relative importance of these crops, however, has not been the subject of sufficient investigation to warrant very definite statements.

Among the methods of studying rates of decomposition, nitrification, humus formation, bacterial development, and carbon dioxide evolution are regarded as most reliable. Holtz and Singleton (4) in their work made comparisons of sweet clover and alfalfa sod decomposition by using nitrification as the indicator of decay. They found that nitrification occurred more rapidly in the soil which had sweet clover roots and crowns incorporated with it than in a soil similarly treated with alfalfa roots and crowns.

Roots alone have not received much attention. Analyses of roots and tops of sweet clover and alfalfa, as given by Hopkins (5), indicate that sweet clover tops are richer in nitrogen than alfalfa tops, but that the reverse is true for the roots. This suggests that if the roots unmixed with crowns or leaf accumulations were considered, different results would be obtained. The quantity of crowns mixed with the roots may be of considerable importance in considering the relative value of these crops as green manures.

In an attempt to throw light upon this soil problem, a study was made of the rate of decomposition of alfalfa and sweet clover roots. In order to tie the results up with other decomposition experiments results were also secured for straw. Carbon dioxide evolution, nitrate accumulation, mold development, and humus formation were used as the index of decomposition.

PLAN OF EXPERIMENT

Experiment 1

In the spring of 1926, after a thorough mixing in quantity, 150-gm. portions of a clay loam soil having a pH of 8 were placed in 500-cc. Erlenmeyer flasks. One per cent of air-dried ground alfalfa roots having a total nitrogen content of 1.6 per cent was added to each of the three flasks. Similar flasks were treated with corresponding quantities of sweet clover roots and with straw having a nitrogen content of 0.87 per cent and 0.42 per cent respectively. Three flasks were used as checks. The moisture content of the soils was brought to 25 per cent dry basis. All of the flasks were kept under laboratory conditions at room temperatures varying from 19° to 21°C. The amount of carbon dioxide evolved daily from these soils was determined every 24 hours for a period of 40 days. The apparatus used to determine the CO_2 evolved from the soil was similar to that described by Waksman (17). A stream of carbon-dioxide-free air was drawn through the flasks into standard KOH solution for a period of 20 minutes every 24 hours. Carbon dioxide was titrated for according to the method described by Emerson (3, p. 119).

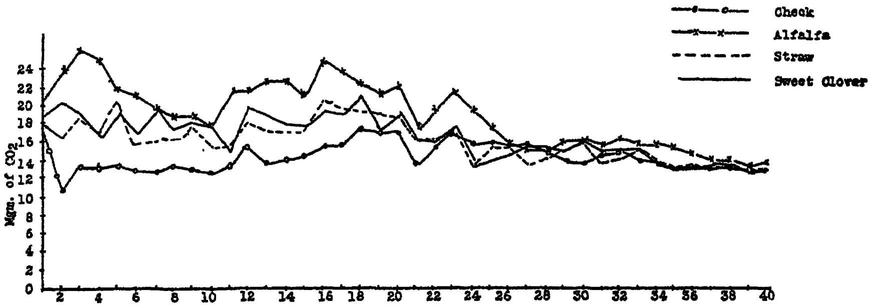

FIG. 1. AMOUNT OF CO_2 EVOLVED DAILY OVER A 40-DAY PERIOD FROM VARIOUS SOILS TREATED WITH DIFFERENT KINDS OF ORGANIC MATTER

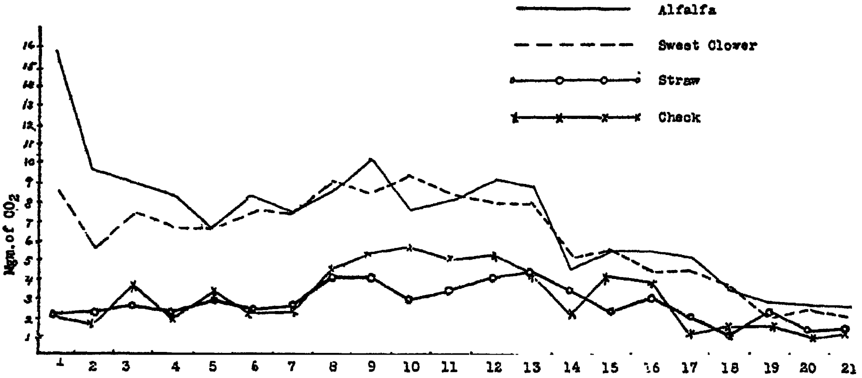

FIG. 2. AMOUNT OF CO_2 EVOLVED DAILY FROM SOIL OVER A 21-DAY PERIOD AFTER BEING INCORPORATED WITH 1 PER CENT QUANTITIES OF DIFFERENT KINDS OF ORGANIC MATTER

Numbers are average of duplicate treatment

The results of this experiment are given in figure 1. The experiment was repeated for a 21-day period. Results are given in figure 2. It will be noted in figures 1 and 2 that the alfalfa-treated soils evolved the greatest amount of carbon dioxide, followed by sweet clover. In figure 1 during the early part of the experiment straw was distinctly above the check and ranked almost with sweet clover. In figure 2, however, the straw and check are almost equal and both fall considerably below either of the legume roots.

The daily variations in the curves are probably largely due to the changes in laboratory temperatures. During spring uniform temperature regulation for the building is difficult. Some of the daily variation in CO_2 may be due to the method of titration used. The indefinite end point as explained by Emerson (3, p. 120) caused more or less uncertainty. All the treatments, however, are subjected to the same influences and despite these fluctuations the curves consistently maintain the relationship which indicates the order of CO_2 evolution to be alfalfa, sweet clover, straw, and untreated soil.

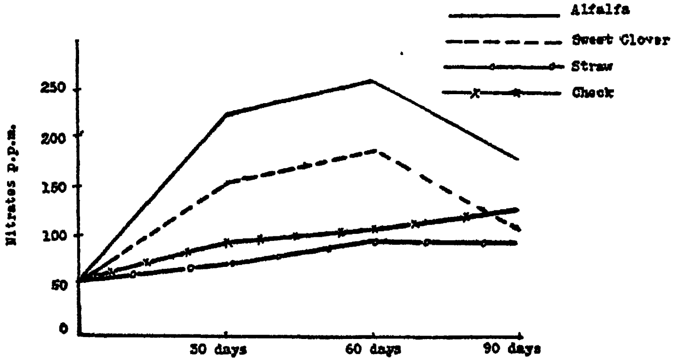

Fig. 3. Effect of Alfalfa and Sweet Clover Roots and Straw Incorporated with the Soil on Parts per Million of Nitrates Accumulated at End of 30-day, 60-day, and 90-day Periods

Result expressed as average of triplicate treatment

In both experiments the greatest differences occurred during the early part of the tests, with a gradual approach of the curves toward the end of the periods, indicating that the vigorous decomposition was nearing completion in about three weeks.

Experiment 2

In this experiment the rate of decomposition as measured by accumulation of nitrates was considered. One-per-cent quantities of dried ground alfalfa roots were mixed with soil having a pH 7.5 reaction. The moisture content was then made up to, and kept at, 25 per cent dry basis. This was repeated with sweet clover roots and straw. These treatments together with the checks were run in triplicate. The pots were incubated in the laboratory at room temperature. Nitrates were determined by the phenol-di-sulfonic acid method, after 1-month, 2-month, and 3-month periods. The tests were running at the same time as those reported in experiment 1. The results are given in figure 3. The experiment was repeated for a 21-day period and the pots were incubated in the greenhouse. Nitrates were determined at the beginning and at the end. Results are shown in figure 4.

The results show more nitrates in alfalfa-treated soils than in any other; or, in other words, when nitrification is used as a measure of decay, alfalfa roots decompose the most readily.

As indicated in figure 4, which experiment was continued for 21 days only, the organic matter depressed nitrification. It is probable that if the experiment had been carried on further there would have been a recovery from the initial depression. Such a conclusion seems justified from a consideration of figure 3.

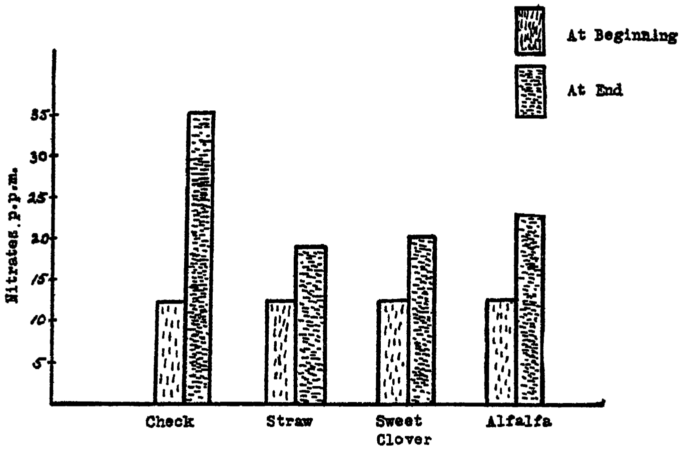

FIG. 4. PARTS PER MILLION OF NITRATES IN SOIL AT END OF A 21-DAY INCUBATION PERIOD AFTER BEING INCORPORATED WITH DIFFERENT KINDS OF ORGANIC MATTER

In previous work by the author (7), it was found that the accumulation of nitrates was a good measure of decay and the greater the accumulation the greater the rate of decay. Holtz and Singleton (4), Waksman (16, p. 509), Wright (18), and Maynard (9) also indicate that the accumulation of nitrates may be taken to indicate the rate at which organic materials decay when incorporated with the soil.

From the results indicated in figures 3 and 4, one would be led to conclude that alfalfa roots decomposed at the greatest rate, followed by sweet clover then by straw.

Holtz and Singleton (4) found sweet clover sod decomposed more readily than alfalfa sod. The sod includes the crowns, roots, and probably the accumulations of leaves. Alfalfa roots alone, as reported by Hopkins (5), contain a considerably higher nitrogen content than sweet clover roots and vice-versa for the tops. The results thus far, in showing alfalfa superior to sweet clover, may seem antagonistic to the experiments of other workers along this line, unless a word of explanation be given. The accumulated leaves and crown with high nitrogen content, mixed with low nitrogen content of the sweet clover roots, probably totals a higher nitrogen content in the sweet clover mixture than with the alfalfa root and crown mixture. This would indicate a narrower C-N ratio than in the alfalfa mixture. The conclusions of Sievers (11) and Waksman (16, p. 509) which have been verified by the author (8) and others are that the narrower the C-N ratio the more readily the material decomposes. This is sufficient to explain why Holtz and Singleton found that sweet clover sod nitrified more readily than alfalfa sod, whereas the results given in figures 3 and 4 show the reverse for the roots alone.

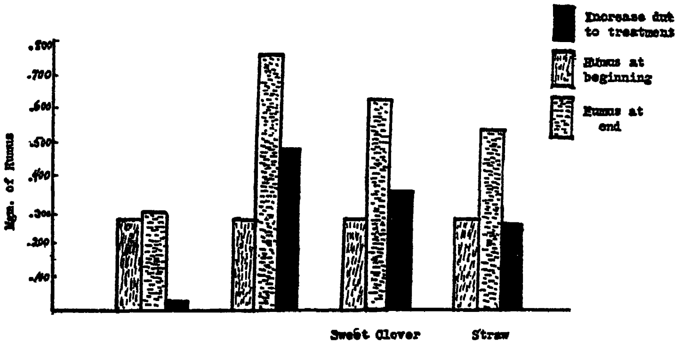

FIG. 5. EFFECT OF ALFALFA AND SWEET CLOVER ROOTS AND STRAW ON AMOUNT OF HUMUS FORMED IN SOIL AT END OF A 3-MONTH PERIOD

Experiment 3

In this test humus was used as the measure of the rate of decomposition of alfalfa roots, sweet clover roots, and straw. The soils treated as in experiment 2 were sampled for humus studies at the same time the samples were obtained for nitrate determinations.

The method used for determining the humus was the one recommended by Waksman (15) after his thorough investigation of all methods (14). The results are given in figure 5.

The results are similar to those for nitrates, shown in figure 3. This was expected because experiments show that the greater amount of humus the greater the accumulation of nitrates. Marshall (6) states that under suitable conditions the accumulation of humus in the soil stimulates nitrification to a very striking extent. The author (7) used humus formation as a measure of rate of decay and found that nitrates increased as humus increased. Carr (2) suggested humus formation as a step in the process of decomposition, the rate of decay being approximately the rate at which humus is produced. Merkle (10) used rate of humus formation as a measure and found a correlation.

Since humus formation appears to be a reliable test of the rate of decay, this experiment is in close agreement with the previous ones in showing that alfalfa roots decompose most readily, followed by clover roots and straw.

Experiment 4

A fourth method for studying the rate of decay is by the activity of the micro-organisms causing the decay. This constitutes the subject of experiment 4.

Soils were treated as described under experiment 2, and samples were obtained at weekly intervals for 6 weeks. Mold counts and identification of mold genera were made from each sample. Each was treated in triplicate, and 3 samples were taken each week. Three plates were poured from each sample, thus making 9 plates for each treatment. The average of each 9-plate group is given in table 1. The agar used was raisin agar as described by Waksman (13).

TABLE 1

Effect on the number of molds per gram of soil of incorporating different kinds of organic material at weekly intervals over a 6-week period

TREATMENT	7 DAYS	14 DAYS	21 DAYS	28 DAYS	35 DAYS	42 DAYS	TOTAL
Alfalfa	27,000	15,000	17,600	15,000	14,300	19,000	107,900
Sweet Clover	15,500	23,000	17,000	15,000	17,000	11,000	98,500
Straw	11,000	17,000	17,500	12,000	12,000	12,000	81,500
Check	6,500	8,500	12,000	10,000	12,000	11,000	60,000

There are many adverse criticisms of the practice of making plate counts of organisms. The limitations have been given by Waksman (16, p. 28). He says:

> . . . . when the results are considered critically and compared carefully it is found that the information obtained from the study of numbers of micro-organisms gives not only an insight into the microbiological population of the soil, but also throws light on soil fertility, particularly when this information is combined with that obtained from the study of physiological activities of soil micro-organisms.

As indicated in table 1, there are irregularities in each week's determination. The differences in the total number of organisms for the 6-week period for each treatment are not very significant. This may be due to a number of factors, among them the use of raisin agar as the medium. According to Waksman (16, p. 19) it is better to use synthetic media in all quantitative work. The slight difference as given indicates that the rate at which alfalfa roots, sweet-clover roots, and straw decay is in the order named.

The genera of the colonies produced on the plates were determined according to the key given by Buchanan (1). The number of each genera was also counted. The results are given in table 2 and indicate that the kinds of organic materials incorporated determine the type of mold genera that develop.

Penicillia predominate in all soil treatments. Mucor, rhizopus, and alternaria predominate in all the alfalfa-treated soils. Cladosporium predominate in straw-treated soils, and aspergillus and penicillium in the sweet-clover-treated soils.

TABLE 2

Effect of different kinds of organic material incorporated with the soil on the development of mold types

The total number for the 6-week period reported

	MUCOR	RHIZOPUS	PENICILLIUM	ALTERNARIA	CLADOSPORIUM	ASPERGILLUS
Alfalfa	38,700	2,800	59,200	2,510	1,760	1,600
Sweet Clover	31,200	900	73,200	250	3,650	4,600
Straw	25,350	2,050	53,750	1,650	5,050	800
Check	16,500	1,000	35,500		2,000	2,500

CONCLUSION

When carbon dioxide evolution, nitrate accumulation, humus formation, and mold counts are used as measures of the rate of decay of alfalfa roots, sweet clover roots, and straw when incorporated with a soil, the materials stand in the order given. The greatest differences occur soon after the materials are incorporated in the soil and thereafter the differences diminish. The predominant types of mold that were found in the soil treated with alfalfa roots are mucor, rhizopus, and alternaria; in soil treated with sweet clover, aspergillus and penicillium; and in soil treated with straw, cladosporium.

REFERENCES

(1) Buchanan, R. E. 1926 Bacteriology, p. 511–547. New York.

(2) Carr, R. E. 1917 Is the humus content of the soil a guide to fertility? *Soil Sci.* 3: 515–524.

(3) Emerson, P. 1925 Soil Characteristics, p. 119. New York.

(4) Holtz, H. F., and Singleton, H. P. 1925 Comparative value of alfalfa and sweet clover on soils in the Lower Yakima Valley. *Jour. Amer. Soc. Agron.* 17: 326–333.

(5) Hopkins, C. G. 1910 Soil Fertility and Permanent Agriculture, 218–224. New York.

(6) Marshall, C. E. 1912 Microbiology, p. 332. Philadelphia.

(7) Martin, T. L. 1921 Decomposition of green manure at different stages of growth. N. Y. (Cornell) Agr. Exp. Sta. Bul. 406.

(8) Martin, T. L. 1925 Effect of straw on accumulation of nitrate and crop growth. *Soil Sci.* 20: 159–164.

(9) MAYNARD, L. A. 1917 The decomposition of sweet clover as a green manure under greenhouse conditions. N. Y. (Cornell) Agr. Exp. Sta. Bul. 394.

(10) MERKLE, F. G. 1918 The decomposition of organic matter in soil. *Jour. Amer. Soc. Agron.* 10: 281–302.

(11) SIEVERS, F. J. 1922 Thirty-second annual report. Report of the division of soils. *Wash. Agr. Exp. Sta. Bul.* 175: 40–42.

(12) SIEVERS, F. J., AND HOLTZ, H. F. 1923 The influence of precipitation on soil composition and on soil organic matter maintenance. *Wash. Agr. Exp. Sta. Bul.* 176: 26–27.

(13) WAKSMAN, S. A. 1922 The growth of fungi in the soil. *Soil Sci.* 14: p. 153–157.

(14) WAKSMAN, S. A. 1926 The origin and nature of soil organic matter or soil "humus": I. Introductory and Historical. *Soil Sci.* 22: 123–162.

(15) WAKSMAN, S. A. 1926 On the origin and nature of soil organic matter or soil "humus": II. Method of determining soil humus in the soil. *Soil Sci.* 22: 231.

(16) WAKSMAN, S. A. 1927 Principles of Soil Microbiology. Baltimore, Md.

(17) WAKSMAN, S. A., AND STARKEY, R. L. 1924 Microbiological analysis as an index of soil fertility: VII. Carbon dioxide evolution. *Soil Sci.* 17: 147.

(18) WRIGHT, R. C. 1915 The influence of certain organic materials upon the transformation of soil nitrogen. *Jour. Amer. Soc. Agron.* 7: 193–208.

THE COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL: II. INFLUENCE OF AGE OF PLANT UPON THE RAPIDITY AND NATURE OF ITS DECOMPOSITION—RYE PLANTS[1]

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It has been shown repeatedly that although the plant continues to assimilate nutrients from the soil until it reaches maturity, the relative (percentage) nitrogen content of the plant reaches a maximum at an early stage of growth; it then begins to diminish, reaching a minimum at maturity, although the total nitrogen content may still increase. This is true not only of the nitrogen but also of certain other plant ingredients. Hunt (4) demonstrated in 1889 that, in the case of various grasses, as the plant matures there is a decrease in the per cent of crude ash, crude fat, and crude protein and an increase in the crude fiber and in the nitrogen-free extract. This is well illustrated from the results of Berry (1), dealing with the composition of oat straw:

	DATE OF SAMPLING					
	June 7	June 21	July 5	July 19	August 3	August 16
Crude protein, *per cent*	16.5	10.6	7.0	4.7	2.8	1.4
Crude fiber, *per cent*	14.1	21.6	24.7	24.7	24.8	28.3

This is true, of course, of the straw more than of the grain as shown by Wilfarth and associates (15), for barley:

	DATE OF SAMPLING			
	May 29	June 17	July 3	July 27
Nitrogen content of straw, *per cent*	2.37	1.16	0.57	0.30
Nitrogen content of grain, *per cent*	....	2.21	1.50	1.42

Unfortunately, most of these studies were limited to the determination of total nitrogen (crude protein), ash, and ether extract (crude fat). Only in exceptional instances was there any attempt made to determine the nature of the non-nitrogenous substances, which are usually included under the general terms of "crude fiber" and "nitrogen-free extractives." The former comprises largely the celluloses, the lignin and the cutins, whereas the latter includes such heterogenous groups as soluble carbohydrates, pentosans and other hemicelluloses, lignins, etc., as seen from a typical analysis reported by König (7).

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Composition of natural materials, on the basis of dry substance

	HAY (MOISTURE, 14.67 PER CENT)	RED CLOVER, BEFORE BLOOM (MOISTURE, 85.95 PER CENT)
	per cent	*per cent*
Crude protein	10.29	19.77
Crude fat	2.76	4.09
Nitrogen-free extractives	50.03	43.26
Crude fiber	31.11	22.82
Ash	5.81	10.08

To understand the nature of the decomposition of plant materials, the difference in the rapidity of decomposition and the liberation of nitrogen and minerals in an available form—a very important problem from the point of view of decomposition of green manures, composting of plant materials and decomposition of stable manures—it is essential to have more definite information on the composition of the plant materials which are usually added to the soil.

It may be sufficient for the present to limit the analysis to the more abundant plant constituents; namely, the carbohydrates, especially the soluble sugars, pentosans, and celluloses; the lignins; the proteins and their derivatives, both water-soluble and water-insoluble; the fats and waxes; and the ash content, without going into a detailed analysis of the less important and less abundant substances found in the plant tissues. The methods previously outlined (14) enable us to carry out such an analysis even in the presence of a large excess of soil.

As to the decomposition of plant materials at different stages of growth, information is even more meagre. The evidence tends to indicate that, at an early stage of growth, the plant constituents decompose in the soil more rapidly than at a later stage or when the plant begins to mature. At the earlier stage of growth, the nitrogen is liberated more rapidly in an available form, namely, ammonia, which is readily changed in the soil into nitrate. It is sufficient to cite the results of Maynard (11) and Merkle (12), who found that the younger the crop plowed under, the more rapidly does it decompose in the soil. Merkle (12) used carbon dioxide evolution, whereas Maynard (11) and Hutchinson and Milligan (5) used nitrate accumulation as indices of decomposition of organic matter. By determining the CO_2 evolution as an index of decomposition, Hill (3) demonstrated that not only do plants decompose more rapidly at an early stage of growth but that there is a definite relation between the nitrogen content of the plant and its decomposition: the higher the nitrogen content the more rapidly does the plant decompose, since the percentage of nitrogen in the plant diminishes with the advance in growth, and its rate of decomposition also diminishes. Since legumes contain more nitrogen than non-legumes, they decompose more rapidly. Drying of green plants was found (16, 3) to retard the decomposition of the plant; the assumption was that, as a result of drying, soluble hemicelluloses change into less soluble forms.

The nature of the controlling factors in these transformations is still to be determined. This is largely because our information on the decomposition of the various constituents of organic materials is still very vague. Certainly the terms "decay," "eremacausis," "fermentation," and "putrefaction," so much beloved by the agronomists, did not tend to clear up the nature of the processes involved. Some of the more recent contributions to the subject tend to indicate that the so-called "humus," or the soil organic matter, consists largely of lignins (2, 8, 13). When organic matter is added to the soil, the various constituents; namely, the water-soluble substances, proteins, pentosans, and celluloses are sooner or later decomposed, whereas the lignins remain undecomposed. This can be demonstrated by an increase in the content of methoxyl, a group characteristic of the lignin molecule, and by an increase of the carbon content of the organic matter. Natural organic matter of plant origin added to the soil contains only 47 to 51 per cent carbon, whereas lignin contains 63 to 64 per cent carbon; soil organic matter ("humus"), however, contains, on the average, 58 per cent carbon.

Even results as definite as these, however, still leave a clearer conception to be desired of the processes involved in the decomposition of organic matter in the soil. This is due to: 1. an insufficient understanding of the rôle of the numerous soil organisms in these processes, 2. a lack of information concerning the part played by the nitrogen of the natural and soil organic matter in the various transformations, 3. the relation between "humus" and the activities of microorganisms. Even König (9) gives the impression that, in the decomposition of manure, "humus" is an intermediary substance between natural organic matter and the final mineralized products (CO_2, nitrate, sulfate, phosphate, etc.)

For the study of the composition and decomposition of plants at different stages of growth, under field conditions, the rye plant was selected. The seeds were planted in the fall in a small plot of ground, which received no special fertilizer treatment. The plants used at each analysis were selected at random from various parts of the plot, then were well mixed, The first sample was taken on April 28, when the plants were only 10 to 14 inches above ground. The plants were sampled again on May 17 when a few of the plants had just begun to form heads, while in most plants the heads were still invisible; in the case of both samples, the whole plant was taken, by cutting it off just above ground. The third sample was taken on June 3, just previous to blooming; in this case the plants (without the roots) were divided into 2 parts: (*a*) heads, and (*b*) stems and leaves; (*a*) and (*b*) were analyzed separately. The

TABLE 1

Composition of rye straw at different stages of growth

SAMPLE NUMBER AND DATE OF SAMPLING	STATE OF PLANT	MOISTURE CONTENT OF PLANT AT HARVESTING	ANALYSIS ON BASIS OF DRY PLANT MATERIAL			COLD WATER-SOLUBLE FRACTION				ALKALI-SOLUBLE FRACTION					PART SOLUBLE IN 2 PER CENT H_2SO_4		PENTOSAN	CELLULOSE	LIGNIN
			Ash content of plant	Total nitrogen content	Ether-soluble fraction	Part of original material	Sugars	Nitrogen in solution	Ash	Part of original material	Lignin (pptd. with hot HCl)	Nitrogen content of lignin	Nitrogen in solution not pptd. by HCl	Reducing sugar in solution	Part soluble	Reducing sugars			
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I April 28	10–14 inches high	80.00	7.66	2.50	2.60	34.24	3.46	0.74	5.08	42.41	3.6	4.20	1.20	14.10	5.81	1.70	16.60	18.06	9.9
II May 17	Just before head begins to form	78.77	5.90	1.76	2.60	22.74	5.98	0.48	4.64	37.48	6.2	3.50	0.92	13.80	6.44	2.35	21.18	26.95	11.8
June 3:																			
IIIa stems and leaves	Just before bloom	57.38	4.90	1.01	1.70	18.16	2.75	0.28	2.44	39.64	7.55	1.40	0.60	19.00	4.30	2.1	22.71	30.59	18.0
IIIb heads		60.23	5.90	2.20	1.95	20.28	2.15	0.58	3.90	44.87	6.90	2.60	0.87	15.12	9.13	2.6	22.67	20.11	16.0
June 30:																			
IVa heads	Plants nearly mature, grains in milk	15.04	3.30	1.26	0.59	4.85	0.71	0.10	0.82	80.60	4.20	2.30	0.74	29.40	6.10	....	11.90	4.59	6.9
IVb stems and leaves		15.00	3.90	0.24	1.26	9.90	2.05	0.07	2.11	44.60	10.20	0.55	0.18	17.10	2.70	....	22.90	36.29	19.8
IVc roots (on ash-free basis)		?*	?*	0.55	0.80	4.66	0.95	0.09	1.04	53.96	9.50	1.95	0.38	20.20	10.40	....	26.55	39.67	17.1

* Since the excess of soil adhering to the roots had to be washed off, the moisture content of the roots could not be determined; the ash content was too high due to some of the adhering soil that could not be removed by washing; the results are, therefore, calculated on ash-free basis.

fourth sample was taken on June 30, with heads fully formed, grains well filled and in milk, just beginning to become hard; in this case the plants were harvested together with the roots, then divided into 3 portions: (*a*) heads, (*b*) stems and leaves, (*c*) roots. The excess of soil adhering to the roots was washed off with a stream of water.

The various samples of plant material were cut into fine pieces with scissors, as soon as they were harvested and brought into the laboratory. The moisture content was immediately determined by the ordinary method of drying at 100°C. to constant weight. Fresh portions of plant material, equivalent to 2 gm. of dry matter, were immediately added to 100-gm. portions of sand or 100-gm. portions of soil in flasks, for the study of the decomposition of the material. The sand cultures received 15-cc. portions of a nutrient solution containing 250 mgm. $(NH_4)_2HPO_4$, 100 mgm. KCl, and 100 mgm. $MgSO_4$ in tap water and were inoculated with a dilute suspension of fresh soil. The moisture content of the soil was brought to an optimum; no nutrients were added to the soil in the case of the first and second samples, one-third of the amount of the nutrients used in the sand cultures was added to the soil containing the third sample, and a full concentration of nutrients was used for the fourth sample in the soil as in the sand cultures. Both sets of cultures were placed in the thermostat connected with the aeration apparatus and incubated for about four weeks at 25–28°C. The carbon dioxide liberated in that period of time was determined at frequent intervals.

TABLE 2

Decomposition of rye at different stages of growth

(2 gm. of dry material added to 100 gm. of sand or soil medium)

DATE OF SAMPLING	NUMBER OF SAMPLE	NITROGEN CONTENT OF MATERIAL	CO_2 GIVEN OFF IN 27 DAYS		AVAILABLE NITROGEN (NH_3-N + NO_3-N) ABSORBED (−) OR LIBERATED (+)	
			Sand medium	Soil medium	Sand medium	Soil medium
		per cent	*mgm. C*	*mgm. C*	*mgm. N*	*mgm. N*
April 28..................	I	2.50	337.7	286.8	+10.1	+22.2
May 17..................	II	1.76	286.5	280.4	+0.8	+3.0
June 3..................	III a	1.01	215.7	199.5	−12.1	−7.5
June 3..................	III b	2.20	261.9	244.8	+5.7	+7.5
June 30..................	IV a	1.26	269.9	273.7	−4.4	−2.1
June 30..................	IV b	0.24	221.4	187.9	−16.0	−8.9
June 30..................	IV c*	0.55	187.0	158.4	−8.1	−9.4

* Root material used in the decomposition was equivalent to 1.67 gm. of moisture-free and ash-free organic matter.

The remaining plant material from each sampling was dried at 80 to 90°C. and subjected to a detailed analysis, according to the procedure outlined previously (14). Table 1 shows the composition of the straw at different dates of sampling. Table 2 shows the rapidity of decomposition of the plant at various stages of growth, as indicated by the evolution of CO_2 as well as by the liberation or absorption of available nitrogen. Tables 3 and 4 show the comparative composition of the organic matter at the beginning and at the end of decomposition processes. In figures 1 and 2 the rates of decomposition of the organic matter in sand and soil media, as indicated by the evolution of CO_2, can be compared.

The results shown in table 1 tend to explain the reason for the difference in the decomposition of plant materials at different stages of growth. When the rye plants are only 10 to 14 inches above ground, 80 per cent of the plant material consists of water, and, of the 20 per cent dry matter, just one-third is water-soluble. The pentosan and cellulose contents are low; the lignin content in the young plant is less than a half of that found in the mature plant. The ash and nitrogen contents are high, but two-thirds of the ash is water-soluble, and a little less than one-third of the nitrogenous substances is water-soluble. The analysis accounts for only 94.98 per cent of the plant constituents, as shown in the following summary:

	per cent
Ash	7.66
Ether-soluble fraction	2.60
Water-soluble fraction (-ash content)	29.16
Lignin	9.90
Pentosan	16.60
Cellulose	18.06
Water-insoluble protein	11.00
	94.98

The remaining 5.02 per cent of the material unaccounted for consists of carbohydrates insoluble in water, outside of the pentosans and celluloses; namely, the starches, pectins, and various hemicelluloses. The existence of these carbohydrates is indicated by the high content of reducing sugars in the fraction soluble in alkali and in hot hydrochloric acid, since boiling with 2 per cent acid resulted in the hydrolysis of these carbohydrates.

The presence of a large amount of water-soluble substance and the high nitrogen content account for the rapidity and amount of decomposition (fig. 1, 2), as indicated by the evolution of CO_2 and by the rapid liberation of nitrogen in an available form, namely, as ammonia and nitrate. Out of 47.7 mgm. of nitrogen present in the 2 gm. of dry material, 10.1 mgm. of nitrogen was liberated in the sand medium, and 22.2 mgm., or nearly one-half of the total nitrogen, in the soil medium, in a period of 27 days. The differences in the liberation of available nitrogen and in the evolution of CO_2 in the sand and soil media can be readily explained by the nature of the organisms which took a more active part in the decomposition processes. The sand, being more open, favored a more rapid development of the fungi; these decompose the organic matter more rapidly, but they store away in their cell substance a greater amount of nitrogen. The soil medium, being more compact, favors a greater development of the bacteria decomposing the various plant constituents; less of the nitrogen, therefore, was reassimilated and more of it liberated in an available form. The soil may also allow a more rapid development of secondary organisms, which decompose the synthesized organic material.

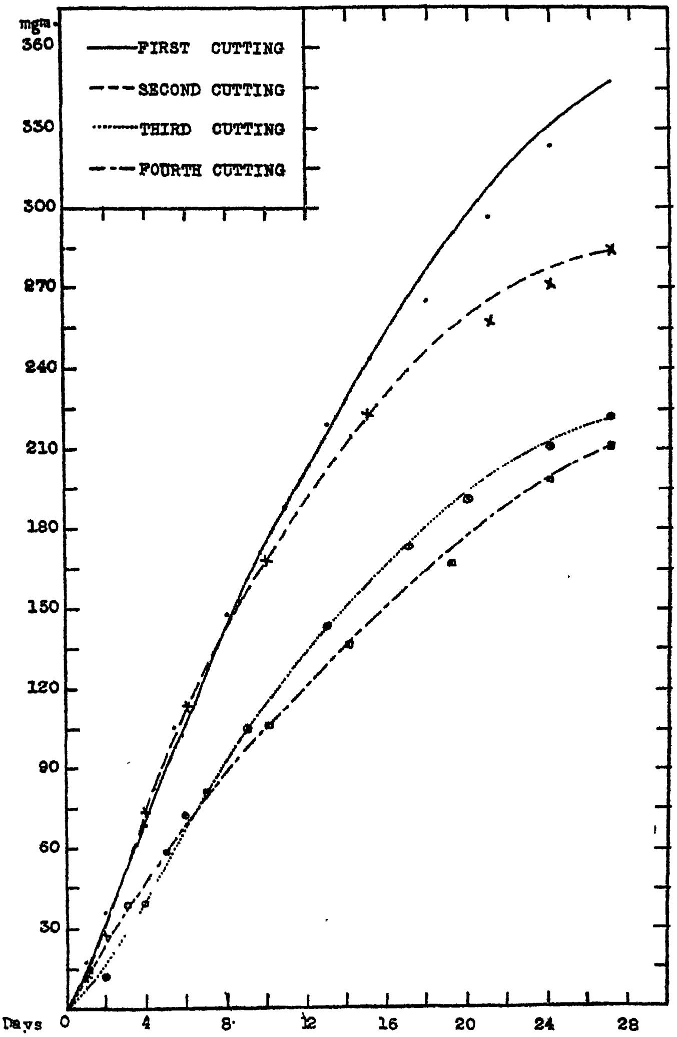

Fig. 1. Influence of Age of Plant (Stems and Leaves) on its Decomposition in Sand Medium as Indicated by the Evolution of Carbon Dioxide

No complete analysis was made at the end of the decomposition period. The water-insoluble organic matter was reduced from 1,347 mgm. at the beginning of decomposition to 300 mgm. at the end of decomposition in the sand medium; in other words nearly all the organic matter has become decomposed and the remaining material is largely synthetic in nature, as seen from the amount of nitrogen stored away by the organisms.

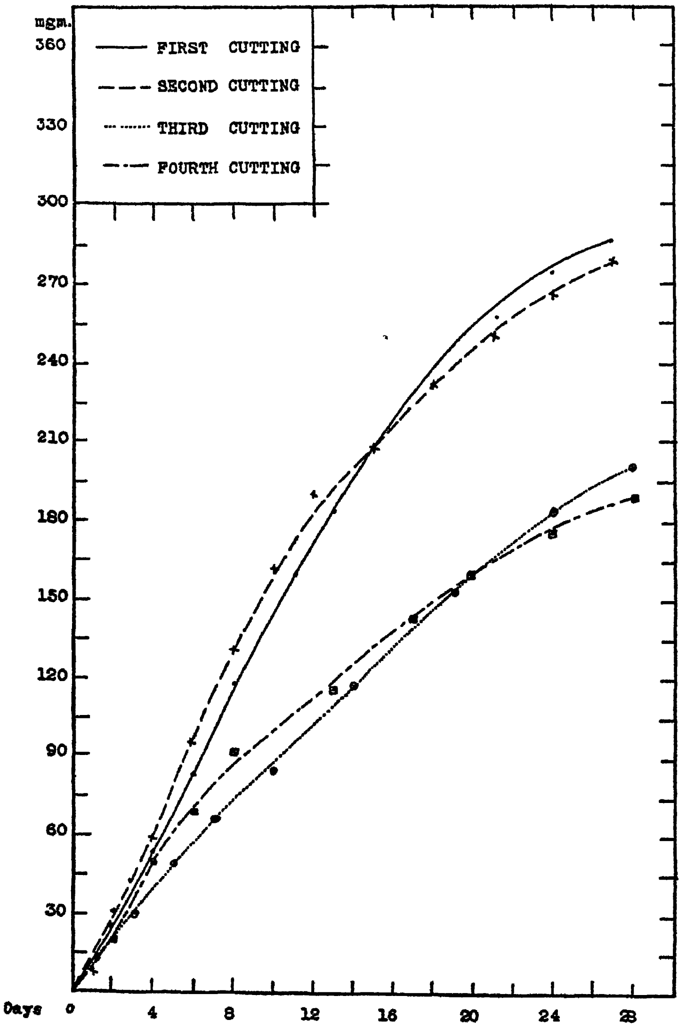

FIG 2. INFLUENCE OF AGE OF PLANT (STEMS AND LEAVES) ON ITS DECOMPOSITION IN SOIL MEDIUM, AS INDICATED BY THE EVOLUTION OF CARBON DIOXIDE

The nitrogen content of the humus of the control was 51.9 mgm. nitrogen for humus extracted from 100 gm. of soil and 78.9 mgm. nitrogen in the humus of the soil receiving 2 gm. of the young rye plants. The nitrogen added in the plant material was 47.7 mgm. Of this 22.2 mgm. was made soluble, the insoluble form accounting fully for the increase in the "humus" nitrogen in the soil.

An examination of the results of the second sampling, taken on May 17, tends to confirm those of the first. The water content of the plant is still very high, being 78.77 per cent of the total fresh weight of the plant. The analysis of the plant accounts for 94.53 per cent of its constituents: ash, 5.90; ether-soluble portion, 2.6, water-soluble (-ash content), 18.10; lignin, 11.8; pentosan, 21.18; cellulose, 26.95; protein, insoluble in water, 8.00. The ash and nitrogen content have diminished; there was a marked reduction in the amount of water-soluble constituents, and an increase in the lignin, pentosan, and cellulose content. The decomposition of this preparation was less rapid than that of the first preparation in the sand medium, but was about the same as in the soil.

TABLE 3

Composition of organic matter at beginning and at end of decomposition

(Sampled May 17)

	AT BEGINNING OF EXPERIMENT	AT END OF EXPERIMENT	
	mgm.	*mgm.*	*per cent*
Organic matter (free from water-soluble substances and ash)	7,465	2,015	27.00
Pentosan	2,050	380	18.54
Cellulose (calculated)	2,610	610	23.37
Lignin	1,180	750	63.56
Protein (insoluble in water)	816	253	31.00
Unaccounted for	8.6 per cent		1.00

The nitrogen content of the plant (1.76 per cent) was just about sufficient to allow the decomposition to proceed rapidly within the given period of time; the microörganisms did not have to draw upon available nitrogen added or present in the soil; there was even a slight liberation of available nitrogen, especially in the soil medium.

The decomposition of the rye plant of the second sampling was carried out in sand and in soil, in a manner previously outlined, but on a larger scale—47.1 gm. of fresh material, equivalent to 10 gm. of dry matter, was added to 200 gm. of sand; 30 cc. of the above medium was introduced and the mixture inoculated with a suspension of fresh soil. At the end of 30 day's incubation, the contents were well mixed and divided into several portions for analysis. Only 2,515 mgm. of organic matter was left, including the water-soluble portion, i.e., 75 per cent of the organic matter has disappeared. The remaining 25 per cent was made up of 7.5 per cent lignin, 3.8 per cent pentosan, 6.1 per cent cellulose (calculated), 5.0 per cent water-soluble material, and 2.6 per cent was not accounted for—this consisted of insoluble minerals, ether-soluble substances, undecomposed protein, and synthesized cell substance. Over 80 per cent of pentosan and over 75 per cent of the cellulose were decomposed, whereas 63.5 per cent of the lignin remained undecomposed. This is brought out in table 3.

It is interesting to note that only one-third of the protein was left; more than two-thirds were decomposed and converted into ammonia and other water-soluble nitrogen compounds. It will be seen later that, with a decrease in the nitrogen content of the plant material the reverse holds true; protein is synthesized rather than decomposed.

The plants sampled on June 3 were separated into: 1. stems and leaves, and 2. heads. The moisture content of the plant was considerably lower than in the previous two samplings. The ash content and especially the nitrogen content have diminished appreciably in the stems and leaves but have increased in the heads. The pentosan content has increased both in the heads and stems, whereas the cellulose content has increased only in the stems. The balance of the analysis can be seen from the following summary:

	STEMS AND LEAVES	HEADS
	per cent	*per cent*
Ash content	4.90	5.90
Ether-soluble fraction	1.70	1.95
Water-soluble fraction (-ash content)	15.72	16.38
Lignin	18.00	16.00
Pentosan	22.71	22.67
Cellulose	30.59	20.11
Protein (water insoluble)	4.40	10.00
Total, *per cent*	98.02	93.01

There is a much better balance in the analysis of the stems and leaves than of the heads, because the starch, which has begun to accumulate in the grains, was not accounted for in this analysis.

A study of the decomposition of the two fractions of the plant has brought out the fact that the heads decomposed more readily than the stems and leaves, no doubt because of the higher protein content, higher starch content, larger water-soluble fraction, and lower cellulose and lignin content.

It is especially interesting to note the available nitrogen balance: whereas the decomposition of 2 gm. of dry material of the heads, both in soil and in sand, resulted in the liberation of available nitrogen as ammonia, the decomposition of the same amount of stems and leaves (the actual amount decomposed was less, as shown by the lower amount of CO_2 liberated), with a lower nitrogen content, resulted in the consumption of nitrogen. When a plant material contains about 1.7 per cent nitrogen, as in the rye of the second sampling, there seems to be about enough nitrogen for the growth of microörganisms which decompose this material more or less completely. When the plant material contains less than 1.7 per cent of nitrogen, as in the case of the stems and leaves of the third preparation, additional nitrogen will be required, before the organic matter is completely decomposed (speaking, of course relatively, since if a long enough period of time is allowed for the decomposition, less additional nitrogen will be needed). If the organic material contains more than 1.7 per cent nitrogen, as in the case of the plants in the first planting and the heads of the third sampling, a part of the nitrogen will be liberated as ammonia, in the decomposition processes. The difference between the nitrogen content of the heads and this hypothetical figure = 0.5 (2.2–1.7) per cent or 10 mgm. nitrogen for the 2 gm. of organic matter used; actually 5.7 mgm. and 7.5 mgm. of nitrogen were liberated as ammonia in the sand and soil media respectively. The difference between the hypothetical figure and the nitrogen content of the stems and leaves was 0.69 (1.7–1.01) per cent or 13.8 mgm. nitrogen for the 2 gm. of plant material used. Actually 12.1 and 7.5 mgm. of nitrogen were consumed in the sand and soil media. Had the decomposition been allowed to proceed further, the results would have approached from both directions the hypothetical figure, and, with prolonged decomposition (of synthesized substances) would have exceeded it.

The rye plants sampled on June 30 were divided into three definite fractions: 1. heads, including the grains; 2. stems and leaves; and 3. roots, from which most of the adhering soil was removed by slight washing. The moisture content of the plant has decreased considerably, being only 15 per cent. The three portions were analyzed and their decomposition studied separately. The following summary gives a balance of the analysis of the three portions of the dry plant:

	HEADS	STEMS AND LEAVES	ROOTS (ASH-FREE BASIS)
	per cent	*per cent*	*per cent*
Ash	3.30	3.90	?
Ether-soluble fraction	0.59	1.26	0.80
Water-soluble fraction (-ash content)	4.03	7.79	3.62
Lignin	6.90	19.80	17.10
Pentosan	11.90	22.90	26.55
Cellulose	4.59	36.29	39.67
Protein (water insoluble)	7.25	1.06	2.88
Total, *per cent*	38.56	93.00	90.62

The incomplete balance in the analysis of the heads is due to the fact that the starch content was not considered in the analysis and the grains contain about 60 per cent of starch as could well be illustrated by the high sugar content of the alkali and acid hydrolyses. The analysis of the stems and leaves shows that the pentosan content has somewhat increased, whereas the lignin and the cellulose have increased considerably. The protein content has diminished very appreciably. An examination of the composition of the roots (on the basis of organic matter free from ash) shows that they are higher in pentosan and cellulose and lower in the ether-soluble and water-soluble portions.

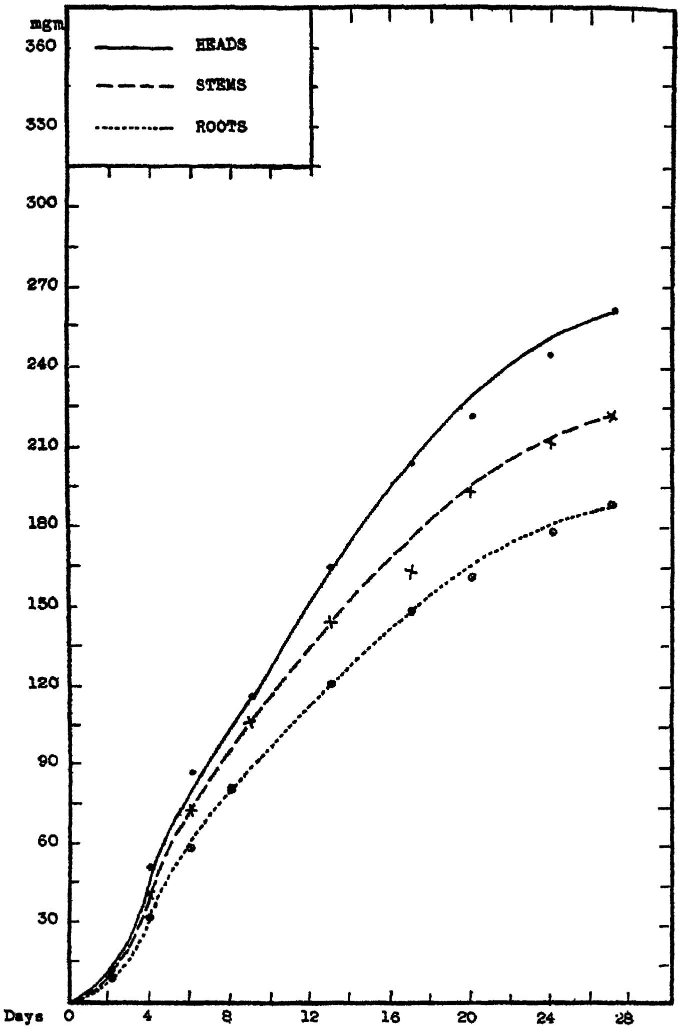

FIG. 3. DECOMPOSITION OF DIFFERENT PARTS OF MATURE PLANTS IN SAND MEDIUM, AS INDICATED BY THE EVOLUTION OF CARBON DIOXIDE

A study of the decomposition (fig. 3, 4) of the three fractions of the nearly mature rye straw shows that the heads decompose very readily, because of the high content of nitrogen and starches; the decomposition of the stems and leaves is similar to that in the third sampling, irrespective of the fact that the nitrogen content of the stems and leaves in the fourth sampling is just about one-fifth that of the third sampling. Since the protein content was rather low in both cases, additional nitrogen was introduced into the sand and soil. The less the nitrogen content of the plant, the greater was the consumption of nitrogen by the organisms which decomposed the plant materials.

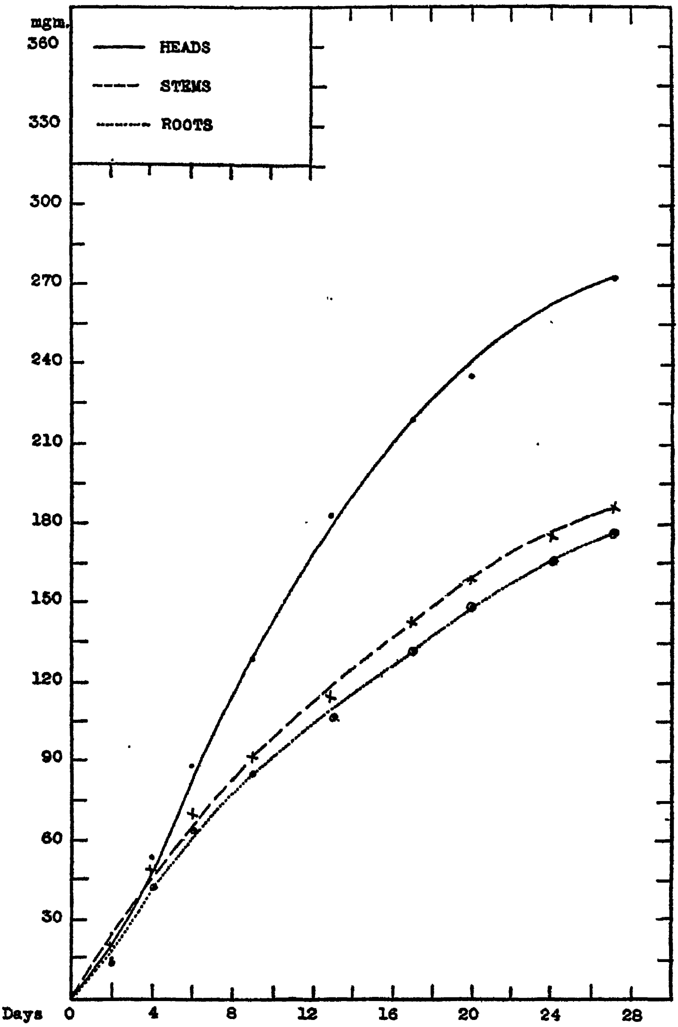

FIG. 4. DECOMPOSITION OF DIFFERENT PARTS OF MATURE PLANTS IN SOIL MEDIUM, AS INDICATED BY THE EVOLUTION OF CARBON DIOXIDE

The roots decomposed rather more slowly than the stems and leaves, probably because of the smaller amount of organic matter introduced (3 gm. equivalent to 1.67 gm. of water- and ash-free organic matter as compared with 2 gm. of stems and leaves equivalent to 1.83 gm. of water- and ash-free organic matter).

Parallel with these decomposition studies, 20-gm. quantities of fresh stems and leaves equivalent to 17 gm. of dry matter, were added to a series of flasks containing 200-gm. portions of either sand or soil. To bring the mixture to optimum moisture, 45-cc. portions of distilled water containing 500 mgm. $(NH_4)_2HPO_4$, 125 mgm. K_2HPO_4, 100 mgm. $MgSO_4$, and 100 mgm. KCl were then added to each flask. The flasks were plugged with cotton and incubated for 2 months. At the end of the incubation period the culture material was taken out of each flask and divided into several definite portions for analysis. The results can be summarized as follows:

In the sand cultures, of the 17 gm. added, there was left, on the average, 8,770 mgm. of the water- and ash-free organic matter. The decomposition did not proceed further because of an insufficient supply of available nitrogen, since only traces of ammonia were left of the 100 mgm. of nitrogen added in the form of ammonium phosphate. The composition of the residual organic matter is given in table 4.

TABLE 4

Composition of organic matter at beginning and at end of decomposition

(Fourth sampling)

	AT BEGINNING OF EXPERIMENT	AT END OF EXPERIMENT	
	mgm.	*mgm.*	*per cent*
Organic matter (free from water-soluble substances and ash)	15,114	8,770	58.03
Pentosan	3,928	1,553	39.54
Cellulose	6,262	2,766	44.17
Lignin	3,403	3,019	88.72
Protein	181	519	286.70
Unaccounted for	10.25 per cent		10.41

Practically all the lignin present in the original plant material was found to be left at the end of decomposition. More than 60 per cent of the pentosan was decomposed, since only 39.6 per cent is left. Of the cellulose, 55.8 per cent is decomposed. Only 181 mgm. of protein, insoluble in cold water, was added; however, at the end of the experiment, 919 mgm. protein was found. These results definitely indicate that, in the decomposition of straw in the soil, the soluble sugars and starches are the first to decompose, followed by the pentosans and celluloses. The pentosans decompose somewhat more rapidly than the celluloses. There is a considerable increase in the amount of protein material, because of the synthesizing activities of the microörganisms, which use the pentosans and celluloses as sources of energy. The lignins are not readily decomposed and the proteins accumulate. These point definitely to the nature of the "humus" complexes in the soil.

The somewhat more rapid decomposition of pentosans than of celluloses has been observed by a number of investigators in the study of the composting of stable manure (10, 6).

The results of the decomposition of the stems and leaves in soil are somewhat similar. Of 3,689 mgm. of pentosan added, only 1,313, or about 36 per cent, was left. Over fifty per cent of the cellulose was decomposed.

The lignin remained largely undecomposed, and has contributed to the formation of humic acid in the soil. The "humic acid" content of the 200 gm. of soil increased from 912 mgm. in the control to 2,076 mgm. As a result of the decomposition of 17 gm. of organic matter, there was an increase of 1,164 mgm. "humic acid" in the soil. The nitrogen content of this humic acid increased from 19.2 mgm. to 33.2 mgm.

SUMMARY

1. A detailed study has been made of the composition of the rye plant at different stages of growth and of its decomposition in sand and in soil by microörganisms.

2. The young plant consists of 80 per cent moisture. A third of the dry portion of the plant is soluble in cold water. The plants contain 2.50 per cent nitrogen, 7.66 per cent ash, 16.6 per cent pentosan, 18.06 per cent cellulose, and 9.9 per cent lignin.

3. With the advance in age of plant, there is a gradual decrease of the nitrogen, ash and fat content, also of the water-soluble portion, and a gradual increase in the content of lignin, pentosan, and cellulose. At maturity, the stems and leaves of the plants contain only 0.24 per cent nitrogen, 9.9 per cent water-soluble substances, 19.8 per cent lignin, 22.9 per cent pentosan, and 36.3 per cent cellulose.

4. The heads and grains of the rye plant contain considerably more nitrogen than the stems, leaves, and roots; less lignin, pentosan, and cellulose; but considerably more starch.

5. The more mature plants decompose more slowly than the younger plants. The rapidity of decomposition depends upon the amount of water-soluble constituents, nitrogen content, lignin content, etc.

6. The rapidity of liberation of nitrogen in an available form depends upon the nitrogen content of the plant and upon the rapidity of decomposition of the plant constituents. The younger the plant, the higher is its nitrogen content and the more rapidly does it decompose; this is accompanied by the liberation of nitrogen in an available form.

7. When the nitrogen content of the plant is about 1.7 per cent, it is just sufficient to cover the requirements of the microörganisms which are active in the decomposition of the plant material within a period of 4 weeks. If the

plant contains more than 1.7 per cent nitrogen, the excess of nitrogen is rapidly liberated in an available form even within the first 4 weeks of decomposition. If the plant material contains less than 1.7 per cent nitrogen, an additional source of available nitrogen will be required before it can be completely decomposed. The lower the nitrogen content of the plant the greater is the amount of additional nitrogen required, or the longer will be the period of time required for the decomposition process.

8. Since there is a very definite ratio between the energy and nitrogen consumption of the microörganisms decomposing the organic matter, it is easy to calculate, with any given amount of plant material and a known nitrogen content, whether nitrogen will be liberated in an available form or additional nitrogen will be required within a given period of time. It is also possible to calculate how much of this nitrogen is required for the decomposition of the plant material and how long it may take before the nitrogen is again made available.

9. In the decomposition of plant materials in the soil, the soluble organic substances are the first to be decomposed, followed by the decomposition of the pentosans and celluloses, the first tending to disappear somewhat more rapidly than the second. The lignins decompose less rapidly than any of the major plant constituents and tend to accumulate in the soil. The plant proteins are readily decomposed and, instead, microbial proteins are synthesized. The latter process accounts for the need in nitrogen for the decomposition of the plant materials.

10. The accumulation of the lignins, which resist decomposition more than the other plant constituents, and the synthesis of microbial nitrogenous complexes account for the increase in soil "humus" as a result of decomposition of natural organic materials. The synthesizing activities of the microörganisms account for the nitrogen content of this "humus."

REFERENCES

(1) Berry, R. A. 1920 Composition and properties of oat grain and straw. *Jour. Agr. Res.* 10: 360.

(2) Fischer, Fr. 1921 Was lehrt die Chemie über die Entstehung und die chemische Struktur der Kohle. *Naturwissenschaften* 9: 958–965.

(3) Hill, H. H. 1926 Decomposition of organic matter in soil. *Jour. Agr. Res.* 33: 77–99.

(4) Hunt, T. F. 1889 Grasses and clovers: effect of ripeness on yield and composition. *Ill. Agr. Exp. Sta. Bul.* 5: 141–164.

(5) Hutchinson, C. M., and Milligan, S. 1914 Green manuring experiments, 1912–1913. *Agr. Res. Inst. Pusa. Bul.* 40: 1–31.

(6) Jegorow, M. 1911 Report of commission on decomposition of manure for 1910. *Ann. Inst. Agron. Moscow* 17: 1–59.

(7) König, J. 1923 Die Untersuchung landwirtschaftlich-wichtiger Stoffe, 5th ed. Berlin.

(8) König, J. 1926 Die Formgebilde der Zellmembran und ihr Verhalten bei der Zersetzung derselben. *Biochem. Ztschr.* 171: 261–276.

(9) König, J., Bach, M., Balks, R., and Hasenbaumer, J. 1926 *Mitt. Deut. Landw. Gesell.* 26: 27.

(10) Löhnis, F. 1910 Handbuch der landwirtschaftlichen Bakteriologie. Berlin.

(11) Maynard, L. A. 1917 The decomposition of sweet clover (Melilotus alba Desr.) as a green manure under greenhouse conditions. N. Y. (Cornell) Agr. Exp. Sta. Bul. 394.

(12) Merkle, F. G. 1918 The decomposition of organic matter in soils. *Jour. Amer. Soc. Agron.* 10: 281–302.

(13) Waksman, S. A. 1926 The origin and nature of the soil organic matter or soil "humus." *Soil Sci.* 22: 122–323.

(14) Waksman, S. A., and Tenney, F. G. 1927 The composition of natural organic materials and their decomposition in the soil: I. Methods of quantitative analysis of plant materials. *Soil Sci.* 24: 275–283.

(15) Wilfarth, H., Romer, H., and Wimmer, G. 1906 Über die Nahrstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. *Landw. Vers. Sta.* 63: 1–70.

(16) Whiting, A. L., and Schoonover, W. R. 1920 The comparative rate of decomposition of green and cured clover tops in soil. *Soil Sci.* 9: 137–149.

A COMPARATIVE STUDY OF THE BACTERIAL FLORA OF WIND-BLOWN SOIL: III. LAKE MICHIGAN SAND DUNES, INDIANA

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INTRODUCTION

This paper is the third in a series of reports, the first two of which were made upon arroyo bank soil and Atlantic Coast dune sand respectively (1, 2). The work herein reported was done during July and August, 1925, upon sand from the dunes at the southern end of Lake Michigan.[1]

METHODS AND MATERIALS

The methods used were the same, in general, as those described in the second paper of the series (2). On account of the chlorination of the Chicago tap water it was replaced in the nutrose agar by distilled water and salts, as suggested by Waksman and Fred (5). Glucose broth was made from beef-extract peptone broth instead of the Difco dehydrated product and 1.5 gm. of beef-extract was used. Nitrate broth was made according to the directions in the Manual of Methods (3).

Soil was collected from two stations, instead of from two places at the same station. Station *a* was the east slope of a rejuvenated second series cottonwood dune in Indiana, about 50 feet above, and approximately 200 feet from, Lake Michigan, due north of Long Lake Station on the interurban line (pl. 1. fig. 1). A low ridge of dunes formed the first series. Station *b* was the west slope of a dune north of Port Chester, Indiana, on the side of a wide wind trough extending inland from the Lake (pl. 1, fig. 2). This station was a few miles east of station *a*. It was also slightly farther from the lake but was at approximately the same altitude.

On account of infection by actinomycetes, coming apparently from the soil outside the laboratory windows, it was exceedingly difficult to obtain accurate results. With a few exceptions at the beginning of the work, the transfer hood was used for all plating and transfers. Several collections of soil were made and many series of plates were poured. Obviously contami-

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[1] The opportunity to do this work was afforded by the courtesy of the staff of the department of botany of the University of Chicago, to whom the writer presents her sincere thanks.

nated plates and questionable cultures were discarded and the averages of plate counts were made from a large number of observations. All cultures were checked. It is possible that this elimination was too severe, since the numbers of organisms per gram of soil were much below what might have been expected for such soil (see table 5).

The object of these surveys was to obtain a general comparison of the bacterial floras of fairly similar soils in various parts of the country. This report, therefore, will be restricted to summaries and averages of data from the soil collected at depths of 6, 12, and 24 inches at both stations in Indiana, arranged in tabular form with similar summaries and averages from the other two soils.

TABLE 1

Annual rainfall for five years previous to the study

LOCALITY	AVERAGE	VARIATION
	inches	*inches*
Tucson, Arizona	13.27	10.37–19.66
Hobart, Indiana*	33.10	28.72–38.20
Sandwich, Massachusetts	43.54	39.08–50.48

* Hobart, Indiana, 5 miles south of Long Lake, was the nearest station from which climatological data could be obtained.

TABLE 2

Summary of the sizes of soil particles

HABITAT	> 2 MM.	1–2 MM.	0.5–1 MM.	0.1 ± –0.5 MM.	< 0.1 ± MM.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Arroyo bank	0.23	0.63	3.33	45.00	50.73
Lake dune	None	None	0.77	98.86	0.40
Marine dune	None	5.37	68.57	25.80	Trace

DISCUSSION

From the tables it will be noted that, with regard to the rainfall and character of the soil, the lake dune habitat was intermediate between the arroyo bank and the marine dune. The arroyo soil was the driest, the most alkaline, and contained the largest amount of soluble salts. Very small quantities of soluble salts were found in the two dune soils, but it is interesting to note that sulfates predominated in the lake dune, and carbonates in the marine dune. In the arroyo soil, nitrates formed the largest percentage.

The figures given in table 5 for the numbers of organisms per gram of the lake dune sand are not considered to be at all satisfactory. As has been mentioned, rigid exclusion of all possibly infected plates probably accounts for

TABLE 3

Summary of the physical characters of the soils

HABITAT	WATER CONTENT	WATER CAPACITY	COMBUSTIBLE MATERIAL	OPTIMUM WATER CONTENT*	RELATIVE WATER CONTENT†
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Arroyo bank	2.01	27.70	1.90	19.37	7.30
Lake dune	2.85	25.00	0.33	17.50	10.72
Marine dune	2.87	23.50	0.27	16.45	12.21

* Optimum water content is taken to be 70 per cent of capacity.

† By relative water content is meant the percentage of capacity present.

TABLE 4

Summary of the chemical characters of the soils

HABITAT	REACTION	TOTAL SOLUBLE SALTS	CO_3	SO_4	Cl	NO_3, ETC.
	pH	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Arroyo bank	8.6–9.0	0.054	0.0100	0.0120	0.0010	0.0350
Lake dune*	7.5–8.0	0.012	0.0005	0.0082	0.0005	0.0028
Marine dune	6.8–7.2	0.007	0.0050	Trace	Trace	0.0020

* The analysis of the soil for the water-soluble salts was made by Miss FrancesSeaver, under the direction of Dr. Mary A. Griggs, Associate Professor of Chemistry, Wellesley College, to whom the writer presents her sincere thanks.

TABLE 5

Average numbers of organisms per gram of fresh soil

HABITAT	DEPTH	TOTAL COUNT	ACTINOMYCETES	FUNGI	BACTERIA, YEASTS
	inches		*per cent*	*per cent*	*per cent*
Arroyo bank	6	401,000	48.50	0.50	51.00
	12	1,898,500	47.47	1.01	51.51
	24	916,500	55.01	0.85	44.04
	All	1,072,000	52.40	0.77	46.81
Lake dune	6	12,950	13.89	2.47	83.63
	12	20,960	10.92	2.00	87.07
	24	18,890	19.58	1.79	78.61
	All	17,600	15.19	2.00	82.80
Marine dune	6	199,900	28.25	2.95	68.78
	12	49,400	14.97	1.82	83.40
	24	31,900	13.79	1.56	89.63
	All	93,700	24.01	2.56	73.53

the low numbers which were, however, rather uniform for the three collections.[2] The three depths are presented because the 12-inch layer had a somewhat higher count than the 6-inch stratum.

TABLE 6

Percentages of colored bacteria, or yeasts, on plates

HABITAT	WHITE	YELLOW	ORANGE	RED	FLUORESCENT*	BROWN
Arroyo bank	88.33	6.39	0.28	1.11	1.39	2.50
Lake dune	83.01	3.07	0.27	0.54	13.09	Few
Marine dune	75.78	7.23	1.57	0.94	14.46	Few

* By transmitted light these were generally dim, colorless colonies which became bluish, greenish, or pearly iridescent by reflected light.

TABLE 7

Summary of the morphology of bacteria in pure cultures

HABITAT	TOTAL NUMBER	COCCUS OR COCCOID	SMALL RODS	LONG RODS		GRAM'S STAIN		
				Spores	None	Positive	Variable	Negative
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Arroyo bank	54	24.10	20.40	42.60	12.90	35.20	....	64.80
Lake dune	89	15.73	46.07	22.47	15.73	31.81	3.40	64.77
Marine dune	61	27.87	42.62	19.67	9.83	38.33	1.67	60.00

TABLE 8

Summary of culture reactions

HABITAT	BACTERIA CAUSING ACID FERMENTATION OF			BACTERIA DIGESTING		BACTERIA REDUCING NITRATES TO NITRITES
	Glucose	Sucrose	Lactose	Gelatin	Casein	
	per cent	*per cent*	*per cent*	*per cent*	*per bent*	*per cent*
Arroyo bank	35.80	28.30	2.20*	72.50	56.50	32.60
Lake dune	24.65	26.39	3.85†	70.83	32.05	18.98
Marine dune	31.58	24.53	?‡	58.49	40.00	23.73

* One form in 24-inch soil.

† Three forms in 24-inch soil.

‡ One form in 6-inch soil gave a questionable reaction, either neutral or very slightly acid.

The percentages of colored forms and the study of pure cultures are presented in tables 6, 7, and 8. The large percentage of fluorescent forms in dune sand from both localities is very interesting, and may be due to the proximity of a large body of water, a situation which was in marked contrast to the conditions in Arizona. In all three soils, white forms were the most abundant and, exclusive of the fluorescent colonies, yellow was the dominant color. No blue, purple, or black forms were found.

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[2] It was planned to have soil collected by exactly the same method, from exactly the same locality and sent to Wellesley, where the plate counts could be checked, but the plans failed of fulfillment and the check could not be made.

One hundred and twenty-four colonies, with as wide a range of characteristics as possible, were selected and studied in pure culture. Eleven cultures died and the remaining 113 formed the basis for the morphological and cultural study. Of these, 10 were actinomycetes; 14, yeasts or yeast-like forms; and 89, bacteria. The cultural study was confined to the last group, the results of which are given in tables 7 and 8, in comparison with similar results from the other soils. In the dune soils the small rods predominated, whereas in the arroyo soil, spore-bearing rods held first place. The percentage of cocci in the lake dune was low. Gram-negative forms predominated in all three soils with remarkably uniform percentages. The culture reactions for the three soils were, in general, rather similar. Roughly, one-third of the forms were sugar fermenters, although there was no gas formed at any time. Very few lactose-fermenting forms were found and these were practically confined to the 24-inch layer. There was less uniformity in the digestion of proteins and the reduction of nitrates. An attempt to group the organisms showed that although no very clear-cut lines could be drawn, the forms fell into certain broad groups.

Arroyo Soil

A. Long rods—29. All rather active forms, of which 26 were white and 3 yellow. They were either digesters or fermenters or both. Nearly all produced spores; about half reduced nitrates and half were gram-negative but there was no correlation between these, or other, characteristics.

B. Short rods—9. Yellow, white, and red forms with yellow predominating. Nearly all were gram-negative. Usually there was digestion of proteins but no fermentation of sugars and little, or no, reduction of nitrates.

C. Cocci—13. Mostly white, gram-negative digesters. There was total lack of fermentation of sugars and reduction of nitrates.

Lake dune soil

A. Long rods—32.
  I. Spore-formers—21. These were all white or transparent.
    a. Active group—11. Mostly transparent and viscid, gram-positive digesters, fermenters, and nitrate reducers.
    b. Inactive group—10. In general these were fermenters but non-digesters; none reduced nitrates.
  II. Non-spore-formers—11. A rather inert group of white, or yellow, forms culturally like I b.
B. Short rods—34.
  I. Active group—9. White forms, with one exception all gram-positive; no uniformity in culture reactions.
  II. Inactive group—25. White or yellow gram-negative forms which, as a rule, did not ferment sugars, digest proteins or reduce nitrates.

C. Cocci—13. Mostly digesters but non-fermenters, with little or no power to reduce nitrates.
  I. Gram-negative, white, yellow, or pink forms—9.
  II. Gram-positive, white, yellow, or orange forms—4

Marine dune soil

A. Long rods—18
  I. Spore-formers—12. Almost exclusively white, gram-positive forms which fermented sugars and digested proteins; only one reduced nitrates.
  II. Non-spore-formers—6. White, yellow, or brown forms which showed no uniformity in characteristics except total lack of reduction of nitrates and a nearly consistent gram-negative reaction.
B. Short rods—25
  I. Active group—12. White or yellow (one brown), gram-positive forms; as a rule, fermenters but non-digesters; about half reduced nitrates.
  II. Inactive group—13. White or yellow (one red), gram-negative forms; as a rule, neither digesters nor fermenters; none reduced nitrates.
C. Cocci—17. White, yellow, orange, or pink forms; mostly gram-positive digesters, but non-fermenters; about one-third reduced nitrates.

If the distribution of the various groups in the three soils is compared, it is found that in the arroyo soil the active long rods were most abundant in the 12-inch layer; the short rods were found chiefly at 6 inches and 24 inches; and the cocci at 6 inches. In the lake dune sand the cocci and active short rods were found in the 6-inch and 12-inch layers only, the active spore-formers almost exclusively at 24 inches, whereas the inactive short and long rods were about evenly distributed in the three layers. In the marine dune sand the short rods (active and inactive groups) were found almost exclusively in the upper two layers and were most abundant at 6 inches; the spore-forming rods were almost entirely restricted to the lower two layers and were most abundant at 24 inches; the cocci were most abundant at 12 inches; whereas the few non-spore-forming long rods were fairly evenly distributed in the three layers.

SUMMARY

1. Lake Michigan sand dunes were intermediate between the arroyo bank in Arizona and the Atlantic Coast dunes of Massachusetts, in rainfall and in physical and chemical character of the soil.

2. On account of certain difficulties in plating, the total numbers of organisms obtained were not considered reliable. The figures given are much below those which might be expected for such soil.

3. There were large percentages of fluorescent forms in both dune soils, probably because of the proximity of large bodies of water.

4. In the dune soils, short rods predominated whereas in the arroyo soil, spore-bearing rods held first place.

5. The three soils were strikingly similar in (*a*), predominance and percentages of gram-negative forms; (*b*), total lack of gas-formers; (*c*), almost

total lack of lactose fermenters; (*d*), the predominance of white forms, with yellow the most usual color; and (*e*),the total lack of purple, blue, and black forms.

6. A classification of the organisms is suggested with a brief discussion of the distribution of the various groups in the three soils.

REFERENCES

(1) Snow, L. M. 1926 A comparative study of the bacterial flora of wind-blown soil: I. Arroyo bank soil, Tucson, Arizona. *Soil Sci.* 21: 143–161.
(2) Snow, L. M. 1927 A comparative study of the bacterial flora of wind-blown soil: II. Atlantic Coast Sand Dunes, Sandwich, Massachusetts. *Soil Sci.* 24: 39–48.
(3) Society of American Bacteriologists. Geneva, N. Y. 1923. Manual of Methods for Pure Culture Study of Bacteria.
(4) U. S. Department of Agriculture, Weather Bureau 1920–1924 Climatological Data, Indiana Section; v. 25 (13); v. 26 (13); v. 27 (13); v. 28 (13); v. 29 (13).
(5) Waksman, S. A., and Fred, E. B. 1922 A tentative outline of the plate method for determining the number of microörganisms in the soil. *Soil Sci.* 14: 27–28.

PLATE 1

Fig. 1. Station *a*—East slope of dune north of Long Lake, Indiana
Fig. 2. Station *b*—West slope of dune north of Port Chester, Indiana

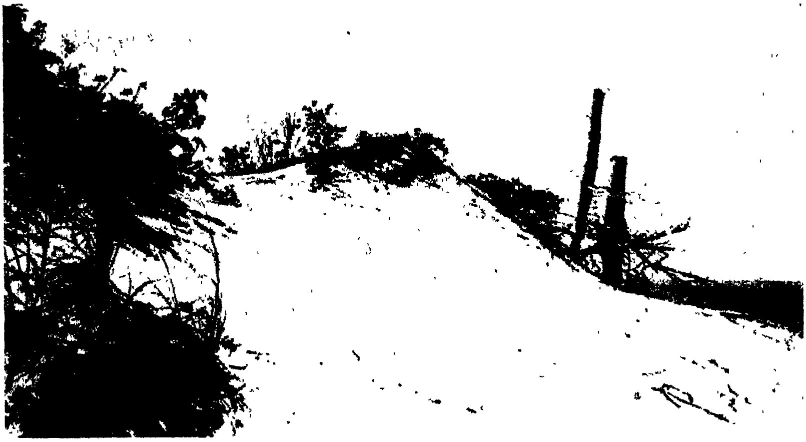

FIG. 1

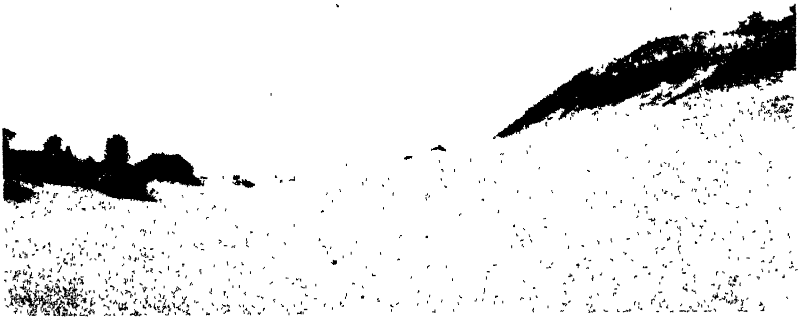

FIG. 2

A BIOLOGICAL MEASUREMENT OF AVAILABLE SOIL POTASSIUM[1]

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Various methods have been used in the past to measure the availability of the more important soil constituents. These methods have involved extraction of the soil with water, carbonated water, salt solutions, and dilute organic and inorganic acids. Mineral acids of relatively high concentrations have also been used. It is now recognized, however, that although certain of these solvents may serve to determine the availability of a single soil constituent, the same will not necessarily hold true in the case of other soil constituents.

At the present time, studies are being made of methods other than those involving extraction with solvents. Among these, that of measuring the availability by means of plants grown on the soils tested, and followed by determinations of the soil constituents absorbed, has been used to a certain extent.

A considerable amount of investigational work has been conducted on the availability of soil potassium, on account of the fact that the ordinary agricultural soils contain sufficient quantities of this element to suffice for the growth of plants for an indefinite period, providing it is rendered soluble. Therefore, a study of the factors influencing the solubility of soil potassium should be of interest both from a practical and scientific point of view.

From the standpoint of plant nutrition, any information pertaining to the quantity of potassium available in soil at any one time, or during a growing season, or as to the form in which it occurs, is of considerable importance, and for these reasons, in part, the following investigation was undertaken.

OBJECT OF THE EXPERIMENT

The object of this experiment was to measure the availability of potassium in two soils of known history, by means of plants grown in direct contact with them, under controlled conditions, and to determine the quantity of this element absorbed. In order to do this a modification of the method of Neubauer and Schneider (4) was employed. The original method is described in detail by Ames and Gerdel (2).

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[1] Published by permission of the Director of the Agricultural Experiment Station as scientific paper No. 431.

EXPERIMENTAL

Description of materials used

The soils used in this study were taken from plots 1 and 4, tier II, of the general fertilizer experiments of the Pennsylvania Experiment Station. These plots have been cultivated and cropped continuously since 1881. Plot 1 received no fertilizer during this time but plot 4 was treated with 200 pounds of muriate of potash biennially, and when sampled had received 18 such applications, the latest of these dressings being applied 14 months prior to sampling. These soils have been described by Frear and Erb (3), who used them for their work on a study of the condition of soil potassium as determined by means of solvents.

The medium for plant growth used throughout this experiment was pure white sand which had been thoroughly washed with tap water and finally with distilled water.

TABLE 1

Composition of nutrient solution

MATERIAL	AMOUNT	VOLUME	APPLICATION
	gm.	*cc.*	*cc.*
$(NH_4)_2HPO_4$	14.4908	8000	10
$CaH_4(PO_4)_2 \cdot H_2O$	25.9389	8000	10
$Ca(NO_3)_2 \cdot H_2O$	68.5225	8000	10
$MgSO_4 \cdot 7\ H_2O$	99.7879	8000	10
K_2SO_4	9.1661	2500	10
$FeCl_3 \cdot 6\ H_2O$	0.1000	250	1

Since buckwheat absorbs potassium in relatively large quantities and grows well under greenhouse conditions, this plant was chosen as the crop to be grown, and in order to insure a good growth, only carefully selected and healthy seeds were planted.

Fifteen seeds were added to each of the jars, and after germination six or seven plants were allowed to develop and the others were removed. The jars contained 2,750 gm. of sand, alone, or with varying quantities of soil.

A complete nutrient solution was added to those jars not receiving a potash addition in the form of soil. Where soil was present, potash was omitted from the nutrient solution. The composition and rate of application of this solution are given in table 1.

Each jar was kept at approximately 14 per cent moisture content with distilled water throughout the experiment.

The amounts of treated and untreated soils added to the various jars and thoroughly mixed with the sand before moisture was added, were 12.5, 25.0, 37.5, and 50.0 gm.

The first application of the nutrients was made on July 12, one week after

TABLE 2

Additions to the quartz sand medium in which plants were grown

JAR NUMBERS	SUPPLEMENTAL TREATMENTS				
	Soil additions		Nutrient additions		
	Plot 1	Plot 4	Kind of solution	Quantity applied	Time of application
	gm.	*gm.*			
1- 2- 3- 4	.....	.....	Complete	Single	Weekly
5- 6- 7- 8	.....	.....	Complete	Single	Semiweekly
9–10	.....	.....	Complete	Double	Weekly
11–12–13–14	.....	.....	K-free	Single	Semiweekly
15–16–17–18	.....	12.5	K-free	Single	Semiweekly
19–20–21–22	.....	25.0	K-free	Single	Semiweekly
23–24–25–26	.....	37.5	K-free	Single	Semiweekly
27–28–29–30	.....	50.0	K-free	Single	Semiweekly
31–32–33–34	12.5	.....	K-free	Single	Semiweekly
35–36–37–38	25.0	.....	K-free	Single	Semiweekly
39–40–41–42	37.5	.....	K-free	Single	Semiweekly
43–44–45–46	50.0	.....	K-free	Single	Semiweekly

TABLE 3

Total yield of dry matter and quantity of potassium absorbed

JAR NUMBER	NUMBER OF PLANTS	WEIGHT OF DRY MATERIAL	WEIGHT OF K ABSORBED	JAR NUMBER	NUMBER OF PLANTS	WEIGHT OF DRY MATERIAL	WEIGHT OF K ABSORBED
		gm.	*mgm.*			*gm.*	*mgm.*
1	6	2.56	77.4	3	7	1.81	68.3
2	6	3.06	76.5	4	7	2.90	69.9
5	6	3.73	136.9	7	7	4.99	161.8
6	6	3.06	121.6	8	7	5.26	159.6
9	6	4.95	157.1	.....	.....	....	......
10	6	3.89	149.1	.....	.....	....	......
11	6	0.77	2.7	13	7	0.79	3.1
12	6	0.57	2.6	14	7	0.64	2.6
15	6	0.99	4.2	17	7	1.19	6.4
16	6	1.23	6.1	18	7	1.63	7.6
19	6	1.66	....	21	7	1.73	......
20	6	1.81	....	22	7	1.82	9.2
23	6	2.28	11.7	25	7	2.13	11.1
24	6	2.14	10.8	26	7	3.16	13.3
27	6	3.55	16.1	29	7	3.23	11.9
28	6	3.73	17.5	30	7	4.06	17.6
31	6	1.05	3.7	33	7	1.11	4.7
32	6	1.06	3.5	34	7	1.06	5.7
35	6	1.06	5.7	37	7	1.49	6.6
36	6	0.90	5.4	38	7	1.06	5.9
39	6	1.44	8.1	41	7	0.96	5.1
40	6	1.27	7.9	42	7	1.24	7.2
43	6	1.46	7.9	45	7	1.38	7.2
44	6	1.28	7.6	46	7	1.32	7.6

planting, followed by successive additions at weekly or semiweekly intervals according to the plan of the experiment.

The jars were weighed daily, care being taken to prevent undue evaporation, by white-washing the greenhouse roof and by keeping the floors well sprinkled with water.

TABLE 4

Availability of potassium in the soils of plot 4 (treated) and plot 1 (untreated)

JAR NUMBER	PLOT NUMBER	WEIGHT OF SOIL	NUMBER OF PLANTS	POTASSIUM REMOVED IN EXCESS OF CHECK	CONCENTRATION OF AVAILABLE K IN SOIL
		gm.		*mgm.*	*per cent*
15	4	12.5	6	1.4	0.0112
16	4	12.5	6	3.3	0.0264
17	4	12.5	7	3.4	0.0272
18	4	12.5	7	4.8	0.0384
19	4	25.0	6	.....	......
20	4	25.0	6	.....	......
21	4	25.0	7	.....	......
22	4	25.0	7	6.4	0.0256
23	4	37.5	6	8.9	0.0237
24	4	37.5	6	8.0	0.0213
25	4	37.5	7	8.3	0.0221
26	4	37.5	7	10.5	0.0280
27	4	50.0	6	13.3	0.0266
28	4	50.0	6	14.7	0.0294
29	4	50.0	7	9.1	0.0182
30	4	50.0	7	14.8	0.0296
31	1	12.5	6	0.9	0.0072
32	1	12.5	6	0.7	0.0056
33	1	12.5	7	1.9	0.0152
34	1	12.5	7	2.9	0.0232
35	1	25.0	6	2.9	0.0116
36	1	25.0	6	2.6	0.0104
37	1	25.0	7	3.8	0.0152
38	1	25.0	7	3.1	0.0124
39	1	37.5	6	5.3	0.0141
40	1	37.5	6	5.1	0.0136
41	1	37.5	7	2.3	0.0061
42	1	37.5	7	4.4	0.0117
43	1	50.0	6	5.1	0.0102
44	1	50.0	6	4.8	0.0096
45	1	50.0	7	4.4	0.0088
46	1	50.0	7	4.8	0.0096

The plants started to bloom on July 30. In order to insure more growth, these blooms were removed and preserved for the final harvest, which was on August 23. At that time the plants were cut close to the sand, placed in large envelopes, and transferred to a drying closet. After the plants were dried, they were ground and preserved for analysis.

At the time of harvesting, an attempt was made to recover the roots. Unfortunately, however, it was found impossible to remove them in their entirety. It was observed, however, that a rather excellent root development was obtained where the plants were grown in the presence of 50 gm. of treated soil, the roots extending to the bottom and throughout the mass of sand and soil, tending to hold it together.

All plants grown in the presence of soil showed pronounced symptoms of potash starvation. Where 50 gm. of the treated soil was used, however, these symptoms did not appear until a week to ten days before harvesting. It was unfortunate that larger quantities of soil were not used in all cases, but the main endeavor in this experiment was to make potassium the limiting factor of plant growth.

The method employed for the determination of potassium in the plants was the same as used by Ames and Boltz (p. 206).

In every case, the entire yield of plants of each jar was taken for individual analysis.

Presentation of data

Forty-six jars were used in the experiment. To some of these were added complete or K-free nutrients in single or double quantities and at intervals of every week or twice a week. To some of the jars were added varying quantities of treated and untreated soils. The supplemental treatments of the jars are given in table 2.

With one exception, two sets of duplicates were run on all individual treatments, one set having 6 plants and the other 7 plants per jar. The total yields of material obtained from each jar, together with the quantity of potassium absorbed are given in table 3.

Previous investigational work on the amount and condition of potassium in each of these two soils, as conducted by Frear and Erb (3) is given below:

AMOUNT OF POTASH	IN TERMS OF AIR-DRY FINE SOIL	
	Plot 1	Plot 4
	per cent K_2O	*per cent* K_2O
Total	3.8210	3.5430
Soluble in hot 1.115 sp. gr. HCl, 10 hours, 1:10	0.3687	0.4092
Soluble in warm 40°C., 0.2 *N* HCl, 5 hours, 1:10	0.0143	0.0301
Soluble in distilled water,		
Flask method, 5 hours, 40°C., 1:10	0.0032	0.0049
Flask method, highest results	0.0046	0.0075
Percolation method, 21°C., 1:10, 4 to 6 days	0.0045	0.0080
In water from clay washing	0.0038	0.0087
Soluble in carbonated water by percolation, 21°C., 1:10, 2 to 5 days	0.0076	0.0140
Soluble in 0.33 *N* NH_4Cl solution, 5 hours, 40°C., 1:10		
First extraction	0.0094	0.0187
Second extraction	0.0020	0.0021

The quantities of potassium absorbed by the six or seven plants of each set of duplicates of the soil treatments after subtracting the quantity found in the plants receiving the K-free nutrients, are given in table 4.

DISCUSSION OF RESULTS

The results obtained by this preliminary experiment, although not conclusive, show that the plants absorbed more potassium from the "treated" soil than from the "untreated." It is also shown that plants must attain a certain condition of growth before they serve as a good index for determining the condition of potassium in soils. In an experiment of this nature, potassium must necessarily be the limiting factor of growth, but better results would doubtless have been obtained if more soil had been used.

Because of the variation normally in evidence where but few plants are grown, there were slight variations in the quantities of potassium absorbed where the plants were grown under the same environmental conditions. No great difference was noted in the quantities absorbed by the six and the seven plants. Calculating the quantity of potassium absorbed by each set of plants, in excess of that absorbed by the check, and averaging the percentages obtained, it was found that the potassium available for plant growth in the treated soil amounted to 0.0252 per cent, and in the untreated soil, 0.0115 per cent. These are practically the same figures obtained by Frear and Erb (3) from their studies of these soils using 0.2 *N* HCl, namely 0.0250 and 0.0118 per cent of potassium, respectively.

SUMMARY

This preliminary experiment suggests a method of attack for determining the availability of potassium in soils. Although the results are meager and do not warrant any definite conclusions, nevertheless, there seems to be a close correlation between the quantities of potassium absorbed by buckwheat plants above the roots and those obtained by the use of 0.2 *N* HCl as a solvent.

REFERENCES

(1) Ames, J. W., and Boltz, G. E. 1915 Tobacco: influence of fertilizers on composition and quality. Ohio Agr. Exp. Sta. Bul. 285.

(2) Ames, J. W., and Gerdel, R. W. 1927 Potassium content of plants as an indicator of available supply in soil. Soil Sci. 23: 199–216.

(3) Frear, W., and Erb, E. S. 1918 Condition of fertilizer potash residues in Hagerstown silty-loam soil. *Jour. Agr. Res.* 15: 59–81.

(4) Neubauer, H., and Schneider, W. 1923 Food consumption of germinating plants and their use in the determination of the fertilizer requirements of soils. *Ztschr. Pflanzenernähr. u. Düngung.* 2 (A): 329–62.

EFFECT OF TREE PRODUCTS ON BACTERIOLOGICAL ACTIVITIES IN SOIL: II. STUDY OF FOREST SOILS[1]

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INTRODUCTION

A description of the forest soil of northern Idaho and of the difficulties encountered in turning this soil into agricultural use has been given (2). This area at the present time is largely timbered, but is being converted to agricultural use as the timber is removed. The first crop after the removal of the timber is as good as could be expected considering the poor physical condition of the soil, but successive crops are even poorer than the first for a period of several years. These soils are known to the homesteader as "turpentine soils," but the actual inhibiting substance has not been found and the cause of unproductiveness is still to be determined. In the previous report, tree products were shown to inhibit ammonia and nitrate accumulation. The object of the present investigation was to study samples of soil taken at widely separated points over the forest area with the hope of throwing more light on the cause of unproductiveness. In this study special emphasis has been placed on nitrogen fixation but other phases of the problem are included.

An effort was made to choose samples representing the various stages of crop history, from virgin timber to many years of cultivation. In no instance was it possible to obtain sufficient data on crop history to make an accurate classification. The number of years of cropping was estimated, which allows grouping into classes that are not definitely bounded. A total of 106 samples of the soil were collected over an area with a radius of approximately fifteen miles. The timber in this area is practically all yellow and white pine, red and white fir, cedar, and larch.

EXPERIMENTAL DATA

Distribution of Azotobacter in forest soils

Unfortunately the method recently developed by Winogradski (3) for determining the approximate number of Azotobacter colonies in the soil was not available when this study was in progress.

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[1] Published with the approval of the Director as Research Paper No. 40, Idaho Agricultural Experiment Station.

[2] Now Research Fellow, Northwestern University Medical School.

[3] Now Soil Biologist, Ohio Agricultural Experiment Station.

TABLE 1

Groups of timber soils with pH and presence of Azotobacter

SOIL NUMBER	GROUP	pH	AZO	SOIL NUMBER	GROUP	pH	AZO
1	IV	7.05	−	46	II	6.88	−
2	IV	....	+	47	III	....	−
3	III	....	−	48	II	6.78	−
4	I	7.05	+	49	I	....	−
5	V	7.05	−	50	II	....	−
6	II	6.68	+	51	I	....	−
7	I	6.36	−	52	II	....	−
8	I	6.27	−	53	I	....	−
9	V	6.15	−	54	II	....	−
10	I	6.07	−	55	II	....	−
11	II	6.95	+	56	V	....	−
12	III	6.90	−	57	II	....	+
13	V	....	−	58	I	6.80	+
14	V	....	−	59	V	....	−
15	I	....	+	60	IV	....	+
16	I	6.07	−	61	III	....	+
17	III	....	−	62	II	6.63	−
18	V	....	+	63	III	....	−
19	I	6.61	+	64	II	....	−
20	I	7.15	+	65	II	....	−
21	V	7.07	−	66	I	....	−
22	I	7.10	−	67	III	....	−
23	I	7.10	−	68	IV	....	−
24	V	6.34	+	69	II	....	−
25	I	5.90	−	70	I	....	−
26	I	5.66	−	71	V	....	−
27	V	6.25	−	72	IV	....	+
28	V	6.54	−	73	III	....	−
29	V	7.42	−	74	II	....	−
30	I	6.32	−	75	III	....	+
31	II	6.70	−	76	III	....	+
32	III	....	−	77	II	6.58	−
33	V	....	−	78	III	....	−
34	I	....	−	79	III	....	−
35	II	....	−	80	V	....	−
36	V	....	+	81	V	....	−
37	V	6.36	+	82	IV	....	+
38	II	6.42	+	83	IV	....	+
39	IV	6.20	−	84	IV	....	+
40	V	....	+	85	III	....	−
41	V	....	−	86	V	6.15	−
42	III	....	+	87	V	6.47	−
43	II	....	−	88	V	6.80	−
44	I	....	−	89	V	6.40	+
45	II	....	−	90	IV	6.30	−
91	I	6.69	−	99	II	....	−
92	V	....	−	100	V	....	−

TABLE 1—*Concluded*

SOIL NUMBER	GROUP	pH	AZO	SOIL NUMBER	GROUP	pH	AZO
93	V	....	+	101	V	....	–
94	II	....	–	102	V	....	–
95	V	....	–	103	V	....	–
96	V	....	–	104	V	....	–
97	V	....	–	105	V	....	–
98	V	....	–	106	V	....	–

Samples of the soils were collected aseptically and brought to the laboratory. Tests were made for the presence of Aztobacter by placing approximately five grams of the soil in each of three 300 cc. Erlenmeyer flasks containing 25 cc. of sterile Ashby's solution. The flasks were incubated at 28°C. until a film had developed. This required approximately ten days. The film was then examined microscopically for the presence of Azotobacter.

The hydrogen-ion concentrations of representative samples of the soil were determined electrometrically.[4]

The data in table 1 show the hydrogen-ion concentration, the presence or absence of Azotobacter, and the group in which the particular soil is classed. Group I represents those soils still bearing virgin timber; group II, secondary timber; group III, cleared, burned, but not cultivated; group IV, cultivated from 1 to 5 years; group V, cultivated for a period of over five years. The boundary between IV and V is by no means accurate.

By assembling the data in table 1 into groups according to stage of cultivation, we have the following results:

GROUP	NUMBER SAMPLES	AZOTOBACTER		PERCENTAGE PRESENT
		Present	Absent	
I	22	5	17	22.7
II	22	4	18	18.6
III	15	4	11	26.6
IV	10	6	4	60.0
V	37	7	30	18.9
	106	26	80	24.5

It will be noted that there is little difference in the incidence of Azotobacter in groups I and II. This is to be expected in that the soil under secondary timber is subject to the same influence as that under virgin timber. There is a greater incidence in the soils cleared and burned and ready for the plow. The small number of soils in group IV and the fact that the distinction between groups IV and V is not accurate, prevent any definite statement relative to the incidence in group IV. The soil cultivated for a number of years showed a smaller incidence of Azotobacter than soils under cultivation for a shorter period, but if we include the two groups in one group of cultivated soils we find the incidence of Azotobacter to be 27.6 per cent, which is greater than the incidence in groups I and II. Azotobacter was found in 26 of the entire 106 samples, an incidence of 24.5 per cent.

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[4] The hydrogen-ion determinations were made by Mr. Louis F. Cady of the Department of Chemistry, University of Idaho.

By arranging the samples in groups according to hydrogen-ion concentration we have the following:

pH	TOTAL	AZOTOBACTER		PERCENTAGE CONTAINING AZOTOBACTER
		Present	Absent	
5.5 -6.0	2	0	2	0
6.01-6.59	17	3	14	17.5
6.6 -7.01	11	3	8	27.2
7.01-7.42	8	2	6	25.0
Not tested	68	16	52	23.5

It is interesting to note that the acidity of these samples is but slight. Of the 38 samples tested, only two were found with a pH below 6.0 whereas 8 were slightly above 7.0. There is apparently no correlation between the acidity of these soils and the presence of Azotobacter when we consider only those with pH above 6.0. There is also no apparent correlation between pH and period of cultivation.

Support of Azotobacter chrococcum

One-hundred-gram portions of samples to 1 to 60, inclusive, were weighed respectively into 500-cc. wide-mouth bottles. The soils were then inoculated with a freshly isolated strain of Azotobacter, and the moisture content brought up to 25 per cent. They were incubated at 28°C. The moisture content was maintained by frequent additions of sterile distilled water. At one period in the experiment, after about six months' incubation, all samples were allowed to air-dry in the bottles and remain in that condition for about two months, after which time the moisture was restored and maintained. Tests for the presence of Azotobacter in the samples were made at regular intervals by removing a small portion of the soil and placing it in sterile Ashby's solution. These tests were continued for a period of 18 months.

Azotobacter was found to be present in 47 of the 60 samples under study after a period of 18 months. The pH of the 13 samples not having Azotobacter after the 18 months, varied from 5.66 to 7.42. The samples in which Azotobacter was not found were 2, 8, 10, 11, 12, 14, 18, 20, 23, 25, 26, 29, and 54. Four of these samples were found to have Azotobacter when first collected, which may be interpreted as indicating that Aztobacter was not a part of the permanent flora of this particular soil or that it was present in such small numbers that its presence could not be demonstrated by the method used. It is apparent from these results that the organism will persist in the majority of these soils when inoculated.

Nitrogen fixation in forest soils

Twenty-four of the samples collected were tested for ability to fix nitrogen when inoculated with Azotobacter. Six 100-gm. portions of each of the 24 samples were weighed into wide-mouth bottles of 500-cc. capacity. Two were inoculated with Azotobacter, 2 were treated with 2 gm. of mannite and inoculated, and 2 were treated with 2 gm. of mannite and 2 gm. of calcium carbonate and inoculated. The moisture content was brought to 30 per cent an additional 2 gm. of moisture being allowed for each added gram of calcium carbonate. All samples were than placed unstoppered in the incubator and the moisture maintained by additions of distilled water and stirring each 4 days. After 4 weeks' incubation they were air-dried and passed through an 80-mesh sieve, and total nitrogen was determined by the Kjeldahl method.

The results, in common with many experiments on nitrogen fixation in soil, were inconsistent and not sufficiently complete for definite conclusions. Samples 26, 27, 30, 37, and 88 fixed amounts of nitrogen varying from 2 to 22 mgm. per 100 gm. of soil in the absence of calcium carbonate, and 6 to 36 mgm. per 100 gm. in the presence of calcium carbonate. Four of these samples apparently contained no Azotobacter when collected. Number 37 contained Azotobacter when collected, and fixed 11.0 mgm. of nitrogen in the absence of $CaCO_3$, and 36 mgm. in its presence. Three of the samples which fixed nitrogen were in group V and two in group I.

Effect of tree products on nitrogen fixation in solution

Various tree products, such as leaves, needles, cones, bark, and sawdust from the trees common to the timbered soils of the area under study were collected, finely ground, and stored in the laboratory. Ashby's solution for the cultivation of Azotobacter was prepared according to the following formula:

	gm.
Magnesium sulfate	0.2
Monobasic potassium phosphate	0.2
Sodium chloride	0.2
Calcium sulfate	0.2
Calcium carbonate	5.0
Distilled water	1000.0

This solution was dispensed 100 cc. per Erlenmeyer flask of liter capacity. The tree products were then added to each of 3 flasks in the amounts indicated in table 2 and allowed to remain several days to become thoroughly saturated. Mannite was then added to each flask at the rate of 15 gm. per liter. Each

TABLE 2

Effect of tree products on nitrogen fixation in solution

TREATMENT			N FIXED PER 100 CC. MEDIUM	RELATIVE FIXATION CONTROL AS 100 PER CENT
		per cent	*mgm.*	*per cent*
	None		7.3	100
Ash	Sawdust	1.0	7.3	100
		2.0	7.5	102.7
		3.0	6.8	93.2
	Leaves	1.0	9.1	124.6
		2.0	10.9	149.4
		3.0	10.7	146.6
Cedar	Sawdust	0.5	5.09	69.7
		1.0	3.05	41.7
		2.0	1.95	26.7
	Leaves	0.5	7.8	106.9
		1.0	5.4	74.0
		2.0	4.8	65.8
Larch	Sawdust	1.0	7.1	97.3
		2.0	6.9	94.5
		3.0	4.5	61.6
	Needles	1.0	5.8	90.4
		2.0	4.2	57.5
		3.0	4.0	54.8
Maple	Sawdust	1.0	5.8	90.4
		2.0	6.9	94.5
		3.0	8.3	113.7
	Leaves	1.0	2.0	27.4
		2.0	0.5	6.8
		3.0	....	.....
White pine	Sawdust	1.0	6.7	91.8
		2.0	7.0	95.9
		3.0	6.7	91.8
	Needles	1.0	1.0	54.8
		2.0	2.0	27.4
		3.0	3.0	28.7
Yellow pine	Sawdust	1.0	7.6	104.1
		2.0	7.6	104.1
		3.0	6.4	87.7
	Needles	1.0	1.2	16.4
		2.0	1.0	13.7
		3.0	0.7	9.5

TABLE 2—*Concluded*

TREATMENT			N FIXED PER 100 CC. MEDIUM	RELATIVE FIXATION CONTROL AS 100 PER CENT
		per cent	*mgm.*	*per cent*
White fir	Sawdust	1.0	7.3	100.0
		2.0	7.0	95.9
		3.0	7.0	95.9
	Needles	1.0	5.0	68.5
		2.0	4.3	58.9
		3.0	....	.....
Red fir	Sawdust	1.0	6.0	82.2
		2.0	5.1	69.9
		3.0	5.8	79.5
	Needles	1.0	6.6	90.4
		2.0	4.2	57.5
		3.0	....	.....

flask was then inoculated with a soil suspension containing Azotobacter. The surface tension was determined by removing with a pipette 25 cc. of the solution and employing the film method of Fahrenwald (1). The solution was then replaced in the flask and incubated at 28°C. The surface tension was determined in order to check the possibility of a change being a factor in the growth of the organisms. The results indicated that it was not, and the surface tension measurements therefore are not presented. The total nitrogen in the cultures was determined after 2 weeks' incubation. The results are shown in table 2. In the data the amount of nitrogen in the added product is deducted from the total nitrogen found and only that amount fixed is indicated.

It will be seen from the data that some of the products inhibited nitrogen fixation. Among the sawdusts, cedar exerted the greatest inhibiting effect, with red fir and larch next in order. Very little inhibiting effect was noted in the other sawdusts. The leaves and needles exerted the greatest retarding effect, ash leaves being the only ones not inhibiting nitrogen fixation.

Effect of tree products on nitrogen fixation in soil

The products enumerated in table 2 were tested for their effect on nitrogen fixation in soil. Varying amounts of the products were added to triplicate 100-gm. portions of a soil known to be capable of fixing nitrogen. After addition of the product each sample received 2 gm. of mannite and 5 cc. of Azotobacter suspension. The moisture equivalent of this soil was 33.7. Preliminary experiments showed that the optimum moisture content for nitrogen fixation was between 20 per cent and 50 per cent more than the moisture equivalent. The moisture content, therefore, was made up to 45 per cent by weight of the soil, and this was maintained by additions of distilled water every 4 days. The soils were stirred 20 hours after each addition of water. They were incubated 4 weeks at 28°C. and the total nitrogen was then determined by the Kjeldahl method. The results are presented in tables 3 and 4. The data in the tables show the average of triplicate determinations and the amount of nitrogen fixed after deducting the amount added in the product. The controls consisted of 100-gm. portions of the untreated soil incubated with the test samples and the moisture maintained as with the other samples.

TABLE 3

Effect of leaves and needles on nitrogen fixation in soil

NUMBER	TREATMENT		N FIXED PER 100 GM. SOIL
		per cent	*mgm.*
1- 3	None	....	6.3
4- 6	Cedar leaves	1.0	10.9
7- 9		2.0	3.2
10–12		3.0	−0.5
13–15	Yellow pine needles	1.0	4.7
16–18		2.0	12.7
19–21		3.0	7.8
22–24	White pine needles	1.0	1.2
25–27		2.0	8.4
28–30		3.0	−2.0
31–33	Larch needles	1.0	11.1
34–36		2.0	3.9
37–39		3.0	−6.8
40–42	Red fir needles	1.0	16.9
43–45		2.0	22.5
46–48		3.0	18.9
49–51	White fir needles	1.0	−1.9
52–54		2.0	−2.7
55–57		3.0	9.8
58–60	Maple leaves	1.0	4.8
61–63		2.0	2.5
64–66		3.0	0
67–69	Ash leaves	1.0	0.8
70–72		2.0	9.7
73–75		3.0	10.8

It is apparent from the data in table 3 that some of the products inhibited nitrogen fixation. The controls fixed an average of 6.3 mgm. of nitrogen per 100 gm. of soil. One per cent cedar leaves stimulated nitrogen fixation, 2 per cent retarded it, and 3 per cent resulted in an actual loss of total nitrogen. The same may be said for larch needles. White pine needles greatly retarded nitrogen fixation when applied at 1 per cent concentration, stimulated at 2 per cent, and resulted in a loss at 3 per cent. The results from applications of white fir are also inconsistent since 3 per cent stimulated and 1 and 2 per cent each resulted in a loss. Maple leaves brought about a consistent retarding influence. Yellow pine needles and, noticeably, red fir needles resulted in

TABLE 4

Effect of sawdust on nitrogen fixation in soil

NUMBER	PRODUCT ADDED		NET AMOUNT OF N FIXED PER 100 GM. SOIL
		per cent	*mgm.*
1- 3	None	....	6.3
4- 6	Cedar sawdust	1.0	9.9
7- 9		2.0	10.2
10–12		3.0	3.2
13–15	Yellow pine sawdust	0.0	21.5
16–18		2.0	12.0
19–21		3.0	4.5
22–24	White pine sawdust	1.0	16.5
25–27		2.0	9.0
28–30		3.0	5.5
31–33	Larch sawdust	1.0	11.7
34–36		2.0	11.4
37–39		3.0	11.5
40–42	Red fir sawdust	1.0	9.0
43–45		2.0	6.7
46–48		3.0	12.5
49–51	White fir sawdust	1.0	13.2
52–54		2.0	11.1
55–57		3.0	6.5
58–60	Maple sawdust	1.0	22.3
61–63		2.0	14.9
64–66		3.0	4.8
67–69	Ash sawdust	1.0	10.5
70–72		2.0	10.4
73–75		3.0	(−6.4)

a stimulation of nitrogen fixation. The loss of total nitrogen in some of the samples is no doubt from the nitrogen in the added product. The nitrogen contained in the added products in table 3 varied from 9.9 mgm. in the case of 1 per cent red fir needles to 50.7 mgm. in the case of 3 per cent maple leaves.

It is apparent from the data in table 4 that all of the sawdusts stimulated nitrogen fixation when applied in amounts of 1 and 2 per cent, whereas larch, red fir, and white fir stimulated in 3 per cent concentration. None of the sawdusts brought about a marked inhibition, even in 3 per cent applications, with the single exception of ash. The amount of nitrogen in the sawdusts varied from 0.6 mgm. to 3.3 mgm.

Ammonia and nitrate accumulation in forest soils

Thirty-one of the samples of forest soil were tested for ability to accumulate ammonia from blood, and nitrate from ammonium sulfate and blood. Ammonia accumulation tests were made by adding 1 gm. of finely pulverized dried blood to 100 gm. of the soil, bringing the moisture content to 25 per cent, and incubating for 1 week. Ammonia was then determined by distillation from copper flasks. Nitrate accumulation tests were made by adding the nitrifiable substance to 100-gm. portions of the soil, inoculating with nitrifying bacteria, and maintaining the moisture content of the soil at 20 per cent through an incubation period of 4 weeks at 28°C. An additional 2 gm. of water was allowed for each gram of carbonate in each sample receiving calcium carbonate. When blood was employed as the nitrifiable substance, 1 gm. was added, and in the case of ammonium sulfate, a sufficient amount was added from standard solution to obtain 30 mgm. of nitrogen. At the end of the incubation period nitrate was determined by the reduction method using Davarda's alloy.

The results of the ammonia and nitrate accumulation tests, together with the group and pH value are presented in table 5.

Some very interesting facts are brought out by the data in table 5. It will be noted that all the soils are capable of accumulating ammonia from blood. There is considerable variation in this respect, but only 3 samples—16, 25 and 26—are outstandingly low. All 3 of these samples were in group I, which is comprised of virgin forest soils; 2 of these soils were the most acid of those tested. It will also be noted that the soils in group I have a lower rate of ammonia accumulation than those in group V, which are the cultivated soils. Although ammonia accumulation in these soils may not be regarded as high, it is apparent that blood is decomposed with the accumulation of ammonia. It is also apparent that cropping increases the rate of ammonia accumulation.

The most interesting results are found in the nitrate accumulation tests. It is apparent from the data that many of these soils are incapable of converting ammonium sulfate or blood to nitrate, and this condition is not overcome by the addition of lime. The acidity is in general very low, as will be noted from the results of the hydrogen-ion determinations. By arranging the soils in table 5 in the order of minimum nitrate accumulation it is seen that they fall into two fairly well defined groups. One group is unable to convert nitrogen in the form of ammonium sulfate or blood, to the nitrate form, whereas the other group, some members of which are relatively low in nitrifying ability, are capable of accumulating nitrate from these substances. By arranging the soils in these two groups we find that we have on the one side virgin timber soils, which are unable to nitrify, and on the other side the soils which have

TABLE 5

Ammonia and nitrate accumulation in forest soils

SAMPLE NUMBER	GROUP	pH	AMMONIFICATION	NITRIFICATION			
			NH$_3$ per 100 gm. soil	Ammonium sulfate		Blood	
				No Ca	Ca	No Ca	Ca
			mgm. N	*mgm. N*	*mgm. N*	*mgm. N*	*mgm. N*
10	I	6.07	46.5	0	0	0	0
16	I	6.07	16.7	0	0	0	0
25	I	5.90	15.0	0	0	0	0
26	I	5.66	15.3	0	0	0	0
19	I	6.61	37.6	0	0	1.3	0
8	I	6.27	49.6	0	0.4	0.46	0.7
1	IV	7.05	51.9	0	3.6	2.6	0.3
4	I	7.05	41.8	0.3	2.5	3.0	0.4
7	I	6.36	37.8	0	2.5	3.6	4.6
23	I	7.10	24.2	0	1.6	1.7	0
17	III	....	58.5	0	2.2	0	6.7
15	I	....	42.3	0	6.0	7.6	3.7
12	III	6.90	43.7	0.6	5.6	7.6	6.8
22	I	7.10	44.5	0	6.1	8.2	7.2
20	I	7.15	39.6	0	0.6	15.0	13.5
30	I	6.32	33.1	1.2	4.2	4.7	17.7
31	II	6.70	22.7	1.4	8.7	3.8	20.2
18	V	....	....	2.3	2.9	31.2	7.7
27	V	6.25	24.5	6.2	15.0	12.0	18.0
5	V	7.05	50.7	7.1	17.1	22.8	5.0
21	V	7.07	41.0	0.4	2.2	30.0	27.2
24	V	6.34	37.6	4.3	10.9	12.8	48.1
11	II	6.95	93.3	1.0	22.3	17.3	37.8
28	V	6.54	25.8	6.3	20.5	15.4	33.8
29	V	7.42	32.4	6.8	22.3	21.1	51.5
2	IV	....	44.1	9.0	22.5	29.0	36.0
3	III	....	55.0	5.0	26.8	30.0	33.6
6	II	6.68	50.0	1.4	27.3	30.5	51.5
9	V	6.15	56.7	8.5	29.5	30.5	68.0
13	V	....	49.2	8.1	27.3	26.8	58.1
14	V	....	46.1	4.4	24.0	21.2	50.0

A nitrate content of less than 0.3 mgm. N as nitrate per 100 gm. soil reported as 0.

been long under cultivation and have developed this capacity. There are but three soils not in group I which are decidedly lacking in ability to accumulate nitrate. On referring to original notes on these samples it was found that soil 1 had been recently plowed for the first time when the sample was taken, soil 12 had been very recently cleared and burned-over, whereas soil 17 had been cleared for many years but had apparently not been cultivated until recently. Of the 31 soils tested 16 fall in the group representing those incapable of nitrate formation.

Of the 31 samples, 15 show the ability to form nitrate, and all but 5 of these are in the long-cultivated group. When ammonium sulfate is employed as the nitrifiable substance, all are very low in nitrate-forming ability in the absence of calcium carbonate. Nitrate formation from ammonium sulfate in this class is in the majority of cases stimulated by the addition of 1 per cent calcium carbonate. Blood is nitrified to a greater extent than ammonium sulfate and this substance is also generally nitrified at a faster rate in the presence of calcium carbonate.

SUMMARY

One hundred and six samples of forest soils were collected asceptically, and brought to the laboratory.

Azotobacter was found to be present in 24.5 per cent of the samples.

There was no direct correlation between crop history or hydrogen-ion concentration and the presence of Azotobacter.

Azotobacter was found to remain in 47 out of 60 samples inoculated for a period of over eighteen months. Tests were not made after that period.

Five of the 24 samples tested for ability to fix nitrogen when inoculated, and supplied with mannite as a source of energy, fixed definite amounts of nitrogen.

Sawdust, leaves, and needles, were tested for their effect on nitrogen fixation in solution by Azotobacter. The sawdusts exerted very little inhibiting action with the exception of cedar. The leaves and needles exerted a retarding influence.

The retarding effect of these products on nitrogen fixation was not marked in soil and applications of 1 and 2 per cent sawdust resulted in stimulation.

Thirty-one of the samples were tested for ammonia and nitrate accumulating ability. All showed ability to accumulate ammonia and all were decidedly lacking in ability to form nitrate from ammonium sulfate or blood. Sixteen of the soils were not benefited in this respect by addition of calcium carbonate, whereas fifteen were greatly benefited.

Those soils which were not able to form nitrate were largely soils bearing virgin timber, whereas those which formed nitrate were largely soils long under cultivation.

REFERENCES

(1) Fahrenwald, A. W. 1922 The Film Method of Measuring Surface and Interfacial Tension. *Jour. Opt. Soc. and Rev. Sci. Insts.* 6: 722–733.

(2) Gibbs, W. M., and Werkman, C. H. 1922 Effect of tree products on bacteriological activities in soil: I. Ammonification and nitrification. *Soil Sci.* 13: 303–322.

(3) Winogradski, S. 1926 Etudes sur la microbiologie du sol: II. Sur les microbes fixateurs d'azote. *Ann. Inst. Pasteur* 40: 455.

THE EFFECT OF THE HYDROGEN-ION CONCENTRATION UPON THE ABSORPTION OF CALCIUM BY A COLLOIDAL CLAY

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The absorption and exchange of bases resulting from the application of different calcium salts to soils has been very extensively investigated, largely because of the important practical bearing of the problem. In but little of this work, however, has sufficient attention been given to the hydrogen-ion concentration resulting after equilibrium between the soil and the calcium carrier has been reached. In an earlier investigation of the absorption of potassium by colloidal clay (1) it was found that the hydrogen-ion concentration was one of the most important variables in determining the course of the reaction. This work was later extended by using calcium instead of potassium carriers (3). In both of these investigations the course of the absorption or exchange reaction was determined by analyses of the clear supernatant liquid above the coagulated clay. In the acid region the behavior of the two cations was noticeably similar, but in the alkaline region significant differences were obtained. In a recent reinvestigation of the problem using electrodialyzed clay and a potentiometric method for estimating the absorption of the cation, results were obtained with both sodium and barium which confirm the work done with calcium and which seem to explain the apparent discrepancy between the two earlier studies.

This paper includes (*a*) a résumé of the findings on the absorption of calcium at varying hydrogen-ion concentrations, (*b*) a brief presentation of some of the results obtained with electrodialyzed clay by the potentiometric method, and (*c*) an explanation of the apparent discrepancy between the earlier and the more recent work.

EXPERIMENTAL

Effect of pH changes upon absorption of calcium

Sufficient 0.042 *N* $Ca(OH)_2$ and 6.30 per cent colloidal clay sol were introduced into a series of fourteen 250-cc. volumetric flasks to make the mixture 0.03 *N* in Ca and 1 per cent in clay when diluted to 250 cc. Sufficient 0.2 *N* HCl was added to each flask to bring it to the desired pH value and then enough distilled water was added to make the total volume 250 cc. The amounts of HCl required were estimated from an electrometric titration curve of the mixture of clay and $Ca(OH)_2$ with the HCl solution. After thorough mixing, the flasks were allowed to stand for 3 days, when aliquots of the clear supernatant liquid were removed for analysis. The pH value and content of SiO_2, Al_2O_3, CaO, MgO, Na_2O, and K_2O of the colloid-free supernatant liquid was determined by standard analytical methods. The average of the results of these analyses are given in table 1.

In figure 1 the amount of total bases liberated and absorbed is shown as a function of the hydrogen-ion concentration. In this case the amount of bases is expressed in terms of milliequivalents per gram of clay. In the acid region more bases are liberated than absorbed, whereas in the alkaline region more are absorbed than liberated. In this respect the results are in harmony with the earlier work with potassium mixtures. There is one important difference between the results obtained with potassium and with calcium. In the case of the former the absorption curve flattened at pH values above 10, whereas in the case of the latter the curve is steepest in this region. There is no evidence of saturation with $Ca(OH)_2$ at the highest pH value obtainable with a saturated lime-water solution. In most of the earlier work on the absorption of $Ca(OH)_2$ by soils, an increased absorption with increasing concentration of the base has been noted. When the Ca absorbed is plotted against the concentration of Ca remaining in solution, a curve resembling the Freundlich adsorption isotherm is obtained. This has been used as an argument that the absorption of the Ca was purely a physical phenomenon. In such studies

TABLE 1

Amount of different ions absorbed and liberated from 1 gm. of colloidal clay at different pH values when 0.03 N Ca was added

Basic exchange

ABSORBED				LIBERATED			
Number	pH	CaO	MgO	KCl and NaCl	Al_2O_3	Fe_2O_3	SiO_2
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	1.96	−6.28	4.90	11.4	4.2	2.79	4.25
2	1.85	−2.05	5.75	11.9	4.0	4.71	3.30
3	2.61	0.53	5.35	11.0	2.1	0.91	1.00
4	3.81	3.75	5.15	10.0	5.8	0.00	0.25
5	4.13	4.88	5.10	9.3	0.4	0.00	0.30
6	4.75	6.75	5.10	9.8	0.0	0.00	0.20
7	6.35	8.70	4.75	12.2	0.0	0.00	0.40
8	6.80	9.18	4.40	13.8	0.0	0.00	0.15
9	6.90	11.90	3.70	12.7	0.0	0.00	0.05
10	7.00	14.95	2.40	9.1	0.0	0.00	0.10
11	8.00	16.67	0.85	10.1	0.2	0.00	0.00
12	8.60	25.45	0.20	8.8	0.7	0.00	0.00
13	10.54	35.12	0.00	9.4	1.5	0.00	0.65
14	11.41	49.12	0.00	9.4	2.1	0.00	0.70

the pH value was, of course, increased as well as the calcium concentration, but this fact has been commonly overlooked.

The results cited above indicate that the pH value has a very great influence upon the absorption process even when the concentration of the Ca ion is kept constant. This leads one to wonder what part of the increased absorption of Ca noted with increasing concentrations of $Ca(OH)_2$ is due to increases in the concentration of the Ca ion and what part to the increase in the hydroxyl-ion concentration.

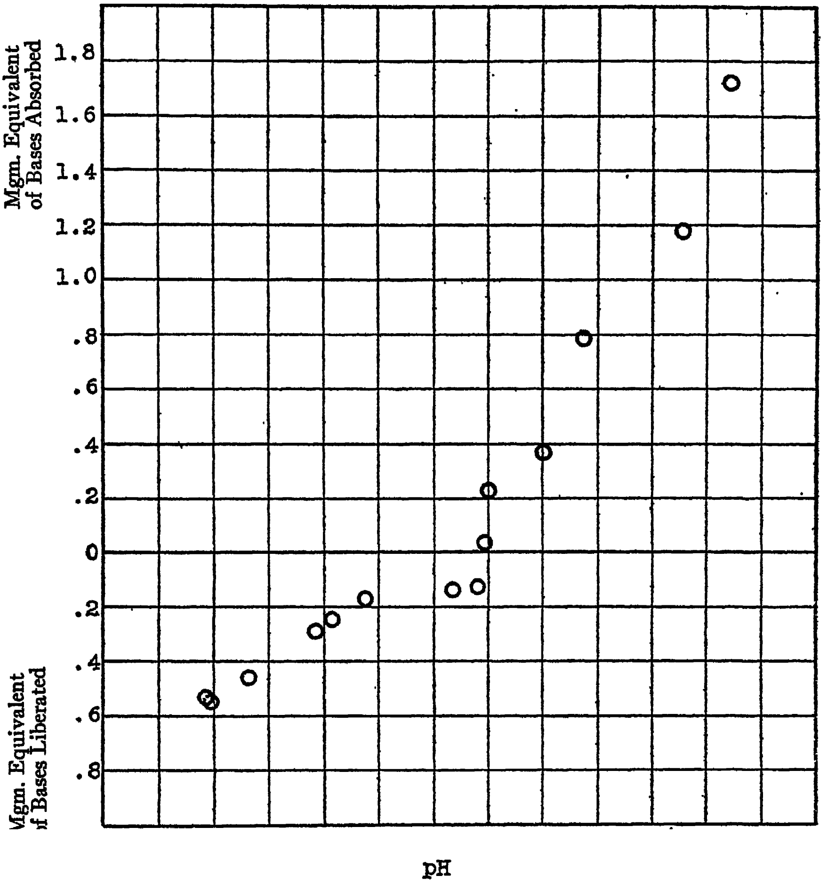

FIG. 1. THE EFFECT OF HYDROGEN-ION CONCENTRATION UPON THE LIBERATION AND ABSORPTION OF BASES BY 1 GM. OF COLLOIDAL CLAY

An answer to this question was sought in four series of experiments similar to the one outlined above, but in each one of which the concentration of the Ca ion was varied. The concentrations of Ca used were 0.0084, 0.0168, 0.0252, and 0.0300 *N*. The variations in pH value were obtained by additions of HCl as in the preceding experiment. In this case the supernatant liquid was analyzed only for calcium. The results of this series of experiments are plotted in figure 2. The experimental error in such a series of experiments is necessarily large, especially with the more dilute solutions. A careful inspection of the curve shows quite conclusively, however, that for the concentrations studied, the effect of the increase of the concentration of the Ca ion upon the absorption of that ion by colloidal clay is negligible in comparison to the effect produced by the OH ion under the same conditions. In fact, these experiments would indicate that the amount of calcium absorbed is practically independent of its concentration at corresponding pH values. The range of concentrations studied was, of course, limited because of the low solubility of CaO in water. The experiment should be extended to the more soluble bases, or possibly by the use of CaO in sucrose solutions.

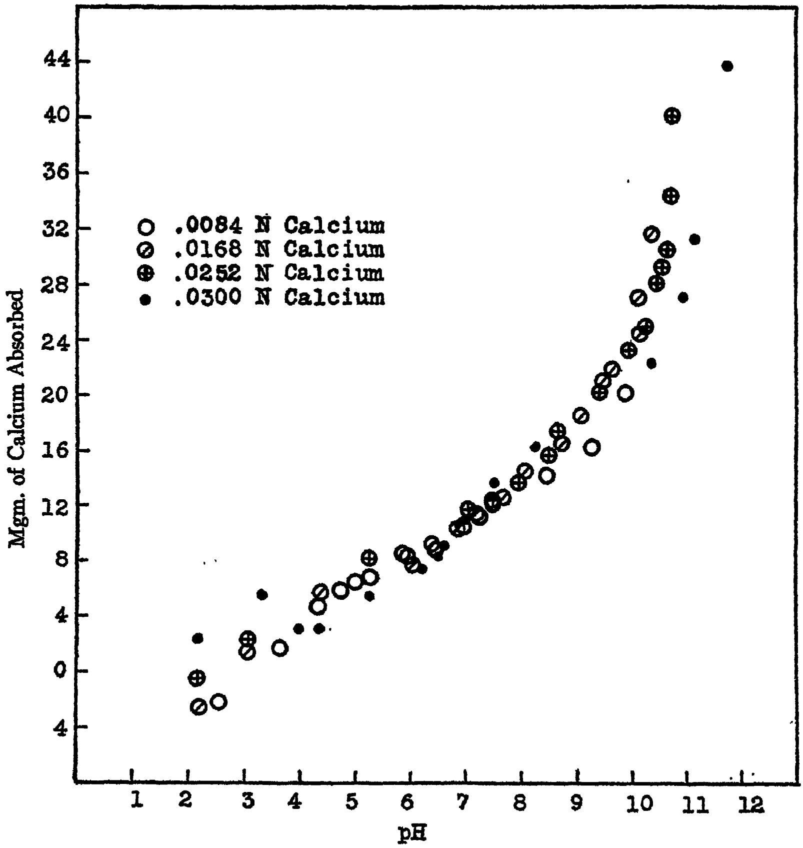

FIG. 2. THE EFFECT OF THE CONCENTRATION OF CALCIUM UPON ITS ABSORPTION AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

As pointed out in the experiments with potassium, the absorption curve flattened at pH values above 10, whereas the calcium curves were steepest in this region. That this difference in the behavior of the two bases is due to secondary reactions taking place in the case of calcium and not to any real difference in the nature of the primary reaction is indicated by the silica and alumina content of the supernatant liquid of the more alkaline mixtures. The

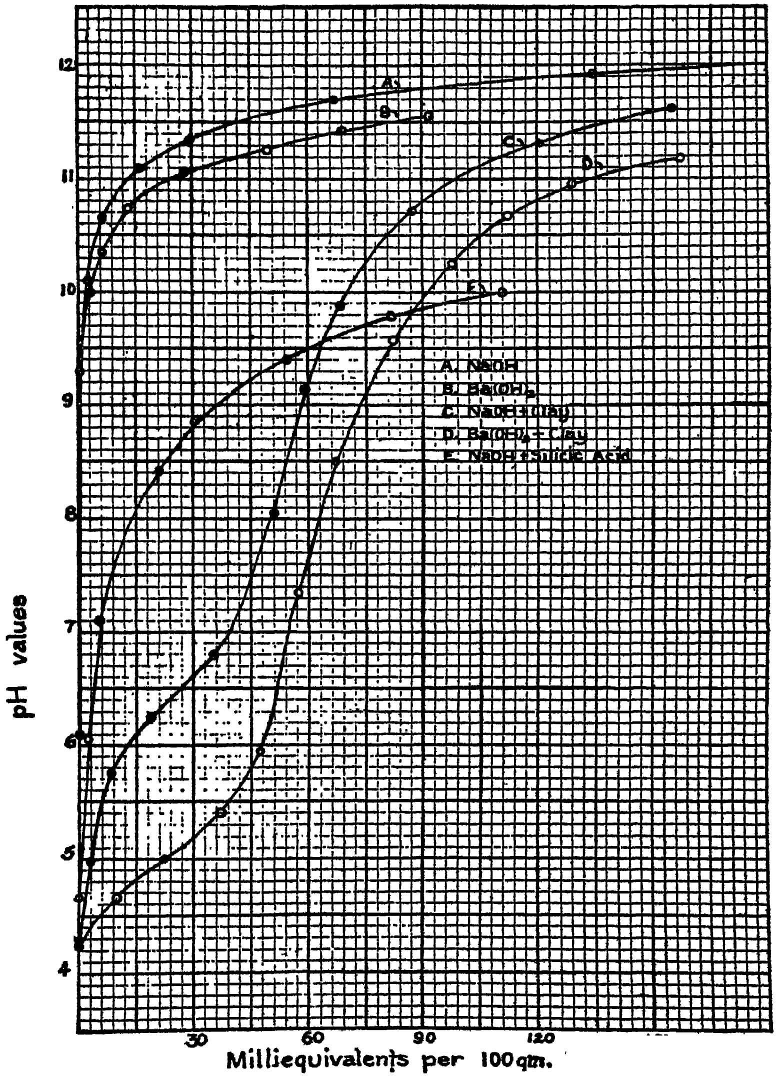

FIG. 3. TITRATION CURVES OF ELECTRODIALYZED COLLOIDAL CLAY MADE WITH THE HYDROGEN ELECTRODE

calcium silicates and aluminates formed by the decomposition of the colloidal clay are not nearly so soluble as the corresponding potassium compounds. Consequently with the technique used in the foregoing experiments the potassium which was combined with the silica and alumina was considered unabsorbed, whereas the corresponding calcium compounds were considered absorbed because of their low solubilities. Further evidence on this point was supplied by the estimation of the absorption by the potentiometric method.

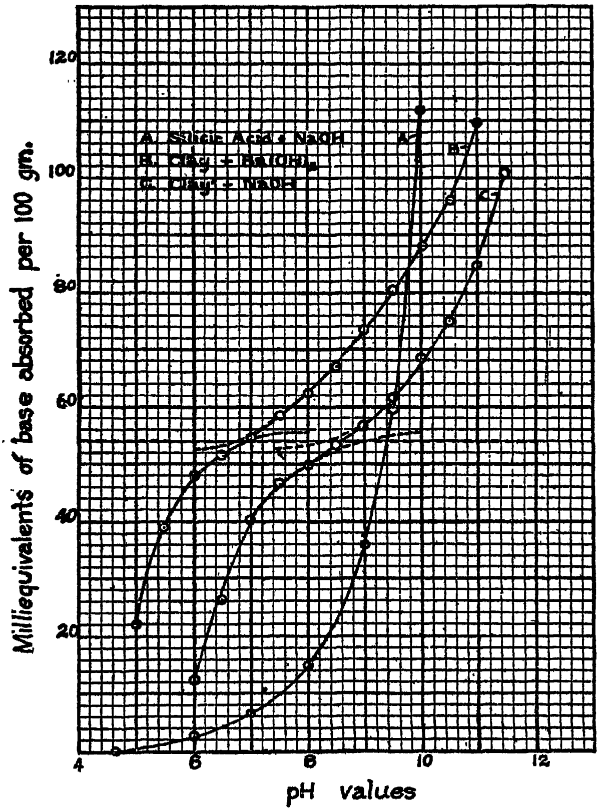

FIG. 4. ABSORPTION OF BASES BY ELECTRODIALYZED COLLOIDAL CLAY AT DIFFERENT PH VALUES

Estimation by potentiometric method of base absorption by clay

Samples of colloidal clay freed from exchangeable bases by electrodialysis were titrated with NaOH and $Ba(OH)_2$ by means of the hydrogen electrode. A second curve was obtained with each base by adding increments to volumes of distilled water equal to that of the clay sol. The difference between the amounts of base required to give any pH value in the presence and in the absence of the clay is a measure of the amount of base absorbed by the clay at that pH value. This method has been extensively used by Loeb (5), Hoffman and Gortner (4), and others for determining the amount of base absorbed by proteins. The titration curves are shown in figure 3 and the absorption curves which were calculated from them are shown in figure 4. In the acid region these curves differ from the absorption curves given. This is largely because in this case the electrodialyzed clay is saturated with hydrogen, which is neutralized by the bases added. Base exchange reactions are therefore eliminated in this case. In the alkaline region the curves are noticeably similar to those obtained with calcium by the analytical method. The sodium and barium curves are of the same type and are almost parallel to each other. The fact that the sodium curve is shifted to the right of the barium curve on the pH axis is due to the greater hydrolysis of the sodium clay. There is no evidence for the slightest difference in the two bases other than this. Evidence of a saturation capacity for bases in the range of pH values studied is also lacking. The titration and absorption curves for an electrodialyzed silicic acid sol shown in figures 3 and 4 offer a clue as to what is taking place in the case of the colloidal clay. The colloidal clay curves seem to have two rather distinct parts, one convex to the pH axis, the other concave. The inflexion point occurs when equivalent amounts of the two bases have been added (54 milliequivalents per 100 gm. of clay), at pH 7 with $Ba(OH)_2$, and at pH 8.5 with NaOH. As shown elsewhere (2) this point seems to represent the limit to which a soil can be saturated with bases by means of a neutral salt. It represents the "saturation capacity" as the term is most commonly used.

This concave portion of the curve is entirely lacking in the silicic acid curve. It resembles the upper part of the clay curves. In other words, silicic acid has practically no power for absorbing bases from an acid medium, but it has an enormous power for absorbing bases in an alkaline medium. It is also well known that Al has a large capacity for absorbing bases from an alkaline medium. That colloidal clay breaks down in strongly alkaline solutions in a manner analagous to that obtained with a sodium carbonate fusion of a soil with the formation of the simpler silicates and aluminates is quite probable. Such reactions would require amounts of base that are enormous in comparison to those commonly dealt with in soil science.

SUMMARY

1. The absorption and exchange of bases resulting from the application of calcium to colloidal clay has been studied between the pH values 2 to 12 by the analytical method. The adsorption curve was steepest in the most alkaline region. There was no evidence of saturation.

2. The amount of calcium absorbed by the colloidal clay seemed to be independent of the concentration of calcium added, when the absorption at the different concentrations was measured at the same pH value.

3. Absorption curves obtained with electrodialyzed clay treated with NaOH and $Ba(OH)_2$ were made up of two rather distinct portions, one covering the

acid range which was convex to the pH axis, while the second covered the alkaline range and was concave to the pH axis. In the first portion of the curve the exchangeable hydrogen was neutralized, whereas in the second a decomposition of the exchange complex with the formation of simpler silicates and aluminates occurred. No evidence of saturation with bases was found at the pH values studied.

REFERENCES

(1) Bradfield, R. 1924 The importance of hydrogen-ion concentration control in physico-chemical studies of heavy soils. *Soil Sci.*, 17: 411–422.

(2) Bradfield, R. The saturation capacity of colloidal clay soils. *Proc. First Internatl. Soils Cong.* Washington, D. C., June, 1927. (Not yet published.)

(3) Cowan, E. W. 1924 The effect of the hydrogen-ion concentration upon the absorption of calcium by colloidal clay. M. A. Thesis, Univ. Missouri.

(4) Hoffman, W. A., and Gortner, R. A. 1924 Physico-chemical studies of proteins. Second Colloid Symposium Monograph, p. 225–240.

(5) Loeb, J. 1922 Proteins, p. 45–48. New York.

MEASUREMENT OF PHYSICAL CHARACTERISTICS OF SOILS[1]

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In soil physics there is a tendency toward the use of generalized descriptive terms in the classification of soils. A soil is spoken of as being heavy, sandy, silty, loamy, sticky, or friable. Indeed a highly technical and standardized nomenclature has grown up among soils investigators. Although these descriptive terms are doubtless very useful, they fail to give complete and exact information. A true knowledge of the physical characteristics of a soil becomes available only when the properties expressed by these descriptive terms can be measured and expressed numerically.

In recent years important steps have been taken to reduce soil science to a more quantitative basis. The development of suitable methods of mechanical analysis marks a great advance in this direction. A number of constants and coefficients such as the coefficient of plasticity, the coefficient of cohesion, the transmission constant, and the hygroscopic coefficient are in use. Some of these are numbers having very complicated dimensions and based on highly standardized methods and apparatus.

The physics department of the Utah Agricultural Experiment Station has undertaken to develop suitable methods of measuring some of the fundamental physical characteristics of soils. A special effort is being made to avoid complicated, standardized apparatus and methods, and to make all units expressible in terms of the fundamental units—mass, length and time.

MEASUREMENT OF COHESION

Cohesion in both wet and dry soil conglomerates seems to be a distinguishing characteristic. It is this tendency of soil particles to stick together that determines how a given soil is to be handled. There have been much speculation and theorizing concerning the effects of structure, chemical composition, presence of colloidal matter, and size of particles on cohesion. To get at these problems, reliable methods of measuring cohesion are needed.

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[2] The author wishes to thank Dr. Willard Gardner and other members of the physics department for the many helpful suggestions and criticisms offered during the course of the experimental work and in the preparation of this article.

The first attempt to measure cohesion at this station was made by measuring the force necessary to draw rods and tubes through the wet soil. Later, attempts were made to get at it by measuring the force necessary to withdraw a glass plate from the surface of the wet conglomerate. These methods not only gave inconsistent results but left doubts in the minds of the investigators as to just what property was actually being measured.

The method finally adopted consists of forcing the wet soil through a glass tube held in a vertical position. The soil forms a column as it issues from the tube and when its weight becomes sufficient to overcome the cohesive forces acting across a right section taken at the extremity of the tube, the column ruptures and the segment falls into a container. A large number of segments are caught and weighed together. When the total weight of the segments and their number are known, the average weight of a segment can be determined, and this, divided by the cross-sectional area of the tube, gives the mean force which when acting across a cross-section of 1 sq. cm. is just sufficient to overcome the internal cohesive forces in the soil.

The errors of measurement by this method are reduced to errors in weighing and in counting the segments. Any errors due to non-homogeneity in the soil sample are compensated for by the large number of segments which go to make up the average, each one giving a measure of the cohesion at a different portion of the sample. The range of water content in the sample is limited only to mixtures so thin that they run through the tube and those so immobile that they cannot be forced through by pressure.

The apparatus is very simple.[3] It consists of a metal cylinder clamped in a vertical position and fitted with a piston. A short metal tube is threaded into the lower end of the cylinder and a glass tube is connected to this with a rubber connection.

Some difficulty was at first encountered in eliminating large air bubbles which got into the soil while it was being stirred with the water before being put into the cylinder. Some of the bubbles were nearly large enough to fill the tube and the segments invariably broke at the points where these occurred. It was found that air bubbles can be eliminated by placing the wet soil in a metal container, the bottom of which is then tapped vigorously on a hard surface; this jars the bubbles to the top of the mixture. The wet soil is then placed carefully in the cylinder, any new bubbles which may have got into it being removed by tapping the cylinder.

To test the usability and accuracy of this method of measuring cohesion a study was made of a series of synthetic soils composed of two extreme types, Greenville loam and Preston clay. The composition of the series was varied in 1 per cent intervals from pure clay ts 65 per cent clay, the moisture content being kept at 62 cc. of water per 100 gm. of dry soil. It was found, as shown by the curves of figure 1, that the cohesion decreases with the clay content in what is shown by a least squares fit to be almost a straight line relationship.[4] At the point in the series corresponding to 85 per cent clay, the mixture became so thin that it was necessary to decrease the water content to 52 cc. per 100 gm. for the rest of the series. It was found that a change in cohesion corresponding to a variation of 2 per cent in soil composition could readily be distinguished by this method.

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[3] The first apparatus used was made from a remodeled automobile grease-gun about 20 cm. long and 6 cm. in diameter. The piston was fitted with a leather washer as a metal to metal fit was found to lodge easily. The glass tube used was 1.2 cm. in diameter.

The relation between cohesion and moisture content for a soil composed of 50 per cent Preston clay and 50 per cent Greenville loam is shown by curve *A* of figure 2. It is believed that every soil has a distinctly characteristic curve of this type which will serve to identify it as well as to give useful information with regard to its behavior when wetted.

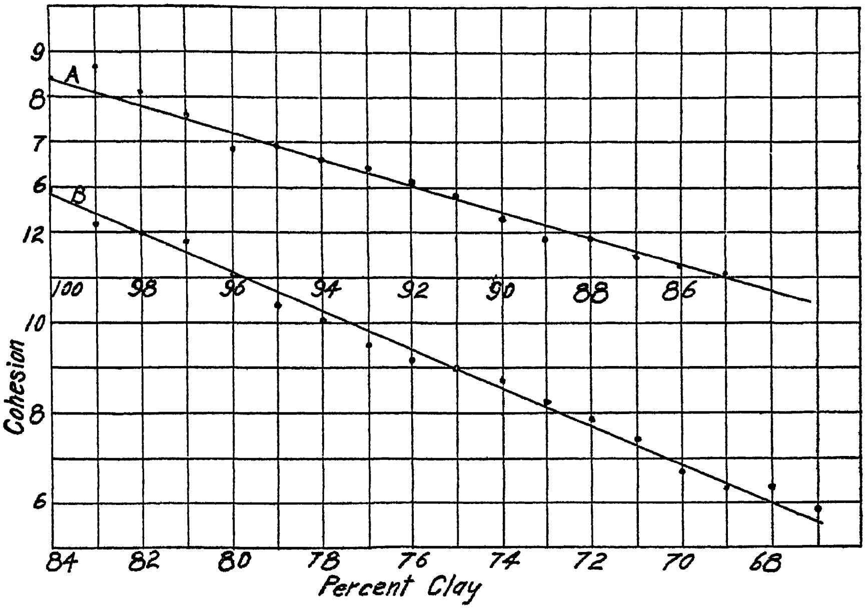

FIG. 1. RELATIONSHIP BETWEEN COHESION AND COMPOSITION OF SOILS

The moisture percentage (dry basis) used in the samples of curve *A* was 62; for those of curve *B* it was 52.

MODULUS OF RUPTURE

A number of attempts to measure cohesion of soils in the dry conglomerate state have been made with some success. Attempts were at first made to test soil cylinders in direct tension but the test specimens were crushed in clamping them in the testing device. Compression tests were tried, but failed to give consistent results.

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[4] In an attempt to fit a second degree curve to the first series, the coefficient of the second degree term came out 0.0001913.

An indirect method of measuring the cohesive properties of soil conglomerates by means of the modulus of rupture has been found to be advantageous. Test specimens of soil are made in the form of cylinders by forcing the soil through a vertical tube while it is in a wet plastic state. As the column issues from the tube it is cut into cylinders with a wire and allowed to stand on end on a glass plate until dry. The cylinders are then tested as simple beams with a concentrated load at the mid span as shown by the diagram of fig. 3.

An expression for the maximum tensil unit stress in the test specimens may be derived from simple considerations of stresses in a beam. When the cylinder is stressed by the application of a load at the mid point the material composing the lower portion is under tension and that composing the upper portion is under compression. Since non-ductile brittle materials like soil conglomerates are weaker in tension than in compression, the cylinder ruptures when the unit tensil stress in the lower portion of the cylinder becomes great enough to exceed the cohesive forces exerted by the soil particles.

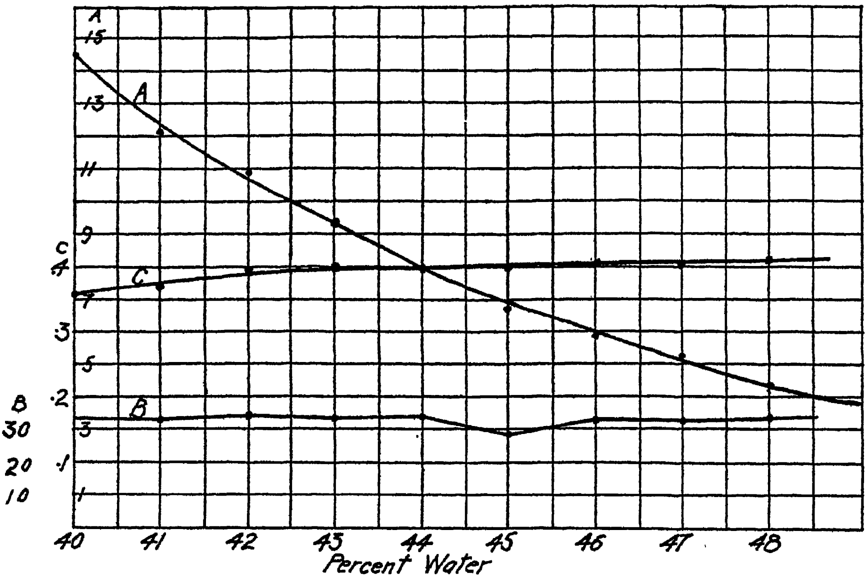

FIG. 2. CURVES SHOWING RELATIONSHIPS BETWEEN MOISTURE CONTENT AND (*A*) COHESION, (*B*) MODULUS OF RUPTURE, (*C*) SHRINKAGE

The magnitude of the cohesive force may be found from the well-known relation $S = \frac{Mc}{I}$ which is the so-called flexure formula and comes from the theory of stresses in beams, where S is the unit tensil or compressive stress,

M the bending moment, C the distance from the horizontal centroidal axis of the cross-section to the point at which the unit stress is desired, and I is the moment of inertia of the cross-section about the centroidal axis. This relation is only valid for cases where the unit deformation in the material is proportional to the unit stress. When the elastic limit of the material is exceeded, the formula does not give correct values of S. It is generally conceded, however, that such materials as soil conglomerates, rupture before the elastic limit is reached, that is, before the strain becomes sufficient to produce permanent deformation.

For cylindrical test specimens stressed to the breaking point, the expression for the cohesion per unit cross sectional area becomes $S = \frac{l_2 l_3 W}{l_1 \pi r^3}$, where r is the radius of the test specimen and l_1, l_2 and l_3 are lengths as designated in figure 3. This value for S is commonly called the modulus of rupture of the material.

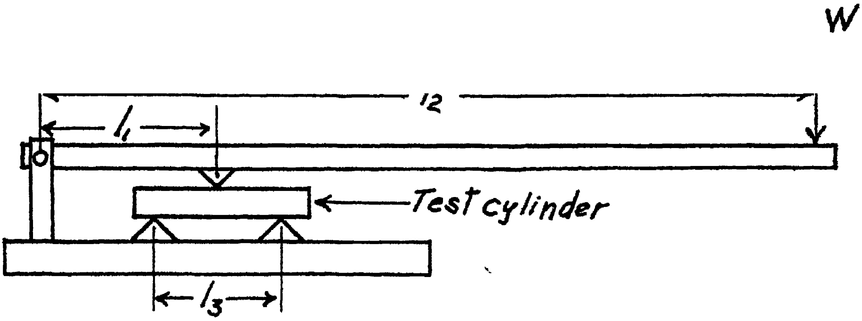

FIG. 3. DIAGRAM OF APPARATUS FOR MODULUS OF RUPTURE DETERMINATIONS

The quantity $\frac{l_2 l_3}{l_1 \pi}$ is a constant of the apparatus depending on the placement of the fulcrums; designating it by K, the expression for the modulus of rupture becomes $S = \frac{KW}{r^3}$.

In the apparatus used, l_1, l_2 and l_3 were about 70, 20, and 2.5 cm. respectively. The force W was applied by hanging a bucket on the lever and slowly running water in until the specimen broke. The fulcrums, especially the lower ones, are slightly rounded and made smooth where they come in contact with the test cylinder. This allows the cylinder to slip slightly and thus eliminates longitudinal forces which would introduce errors.

The results of modulus of rupture determinations made on a series of synthetic soils ranging from 100 per cent Preston clay to 100 per cent Greenville loam are shown by graph A of figure 4. Each point was obtained by taking the mean of six tests. In wetting the soils to make the cylinders, the moisture percentage was so varied as to keep the cohesion in the wet soils approximately constant. The relationship between percentage composition and the modulus of rupture appears to be linear, which might be expected. Graph *B* of figure 2 shows the results obtained by using different percentages of water in wetting the soils to make the cylinders. The results indicate that the amount of water used in wetting the soil has no effect on the modulus of rupture of the dry conglomerate.

The great difficulty in the measurement of cohesion in dry conglomerates seems to be not so much in the accuracy of the determination as in controlling the many physical conditions affecting this property. It is indeed difficult to make two test specimens of the same soil which have exactly the same modulus of rupture. The unavoidable irregularities inherent in soil makeup and lack of control of conditions are responsible for the chief difficulties. When treated by the methods of statistics, however, the results obtained seem to be very promising. The method has the advantage that no standardized apparatus is necessary and that the results are expressed in the intelligible units of grams per square centimeter; this cannot be said of the so-called "point test" and others which are widely used.

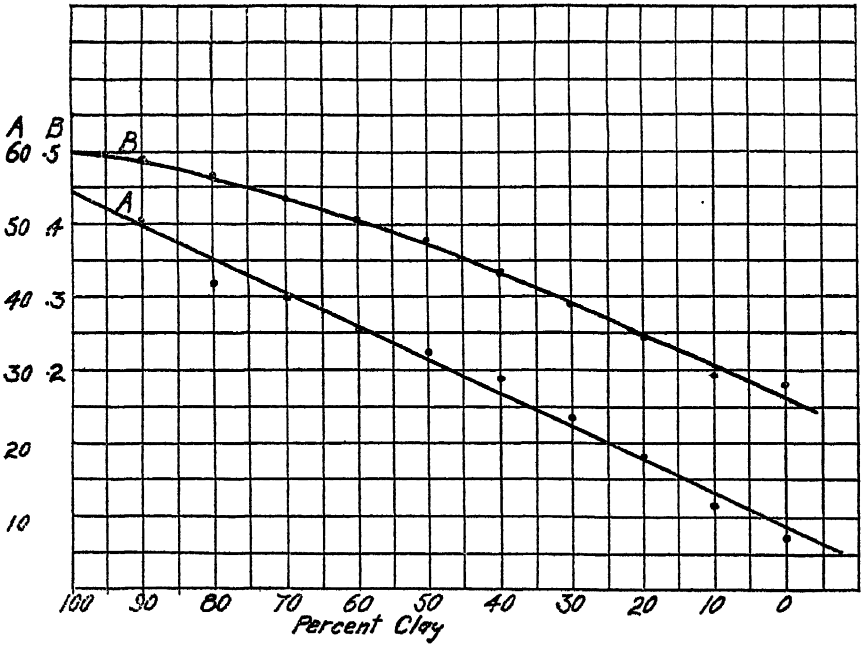

FIG. 4. CURVES REPRESENTING RELATIONSHIP BETWEEN COMPOSITION AND (*A*) MODULUS OF RUPTURE AND (*B*) SHRINKAGE

SHRINKAGE

In making the cylindrical test specimens for modulus of rupture determinations, a very satisfactory way of measuring shrinkage suggested itself. As the wet soil column is forced out of the tube, two small transverse scratches are placed on it by means of a stiff bristle. The scratches are about 2.5 cm. apart on the soil cylinder and the distance between them is accurately measured with a cathetometer while the wet specimen stands in a vertical position on a glass plate. After drying, the distance is again measured with the cathetometer and the diameter measured with micrometer calipers. The diameter of the wet cylinder is the same as the tube through which it was forced. The shrinkage per unit volume is then given by $C = \frac{v_1 - v_2}{v_1}$ in which v_1 is the volume wet, and v_2 the volume dry. This becomes $C = 1 - \frac{l_2 r_2^2}{l_1 r_1^2}$ where l_1 r_1, and l_2 and r_2 are lengths and radii, wet and dry respectively. Values of C accurate in the third figure are easily obtainable by this method. The determination is not only accurate, but variations in shrinkage in cylinders taken from the same soil sample are found only in the third figure. When the mean of a number of measurements is taken, the third figure becomes reliable. Curves *C* and *B* of figures 2 and 4 respectively, show the results of shrinkage measurements made on the same test specimens from which the modulus of rupture data given above were obtained. The curves show that the shrinkage is a function of the water percentage and the percentage composition.

Since the wet soil must be forced through a tube in making determinations of cohesion, modulus of rupture, and shrinkage, it is possible when the water content is not too high to make all three determinations at once on the same soil sample. The test specimens for modulus of rupture determinations may be marked and the shrinkage measured before they are used. This is very desirable, since it makes possible a study of relationships between these important physical characteristics.

REFERENCES

(1) HILGARD, E. W. 1905 Soils, p. 113. New York.

(2) LYON, T. L., FIPPON, E. O., AND BUCKMAN, H. O. 1915 Soils, Their Properties and Management, p. 170–180. New York.

(3) MILLIKAN, R. A. 1924 The Electron, p. 4. Chicago.

(4) SEELEY, F. B. 1924 Resistance of Materials, p. 83–87, and p. 114–116. New York.

STUDIES ON THE TRANSFORMATIONS OF IRON IN NATURE. II. CONCERNING THE IMPORTANCE OF MICROÖRGANISMS IN THE SOLUTION AND PRECIPITATION OF IRON

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INTRODUCTION

Precipitation of iron under natural conditions may result from any of a large number of changes. Not only may there be a variety of products formed, but the agencies primarily responsible for the reactions may be quite different. Among the reactions which have occupied the interests of microbiologists there stand out prominently the changes which have been ascribed to a group of organisms called "iron bacteria." These forms were so called by Winogradsky (39) not alone because they cause a precipitation of ferric hydroxide but because the reduced form of iron was indispensible for their nutrition; indispensible not only for cell construction but as the source of energy for the vital processes of the cells. Incidentally this transformation from the ferrous to the ferric form results in an accumulation of precipitate of ferric iron. Only bacteria which utilize ferrous iron as a source of energy are properly classified as iron bacteria. This group of organisms, if existent, however, is only one of many agencies which may effect a precipitation of compounds of the metal (13). Strictly chemical changes of inorganic compounds may occur in the absence of microörganisms. Transformations of inorganic compounds may take place indirectly as a result of a modification of the environment by the development of microörganisms. Decomposition of organic compounds of iron may be followed by a similar deposition. In such changes iron plays no rôle of importance in the development of microorganisms. In fact, since the reactions of oxidation and precipitation are reversible, slight modifications of the environmental conditions may not only inhibit precipitation but favor solution.

As emphasized particularly by Winogradsky (40) and Cholodny (5) there is a very clear distinction between organisms included as iron bacteria and those organisms which may be associated with precipitation of iron. Although the iron bacteria are all responsible for precipitation of iron, only a small group of the organisms active in the precipitation of iron may be considered to be iron bacteria.[3] Too frequently the criterion by which organisms were judged to be iron bacteria was the precipitation of ferric hydrate rather than the reactions responsible for the precipitation.

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Whatever may be responsible for them, these transformations of iron become of considerable importance in numerous connections. This is particularly the case in the large deposits of iron ore of aquatic origin (10, 13). Closely associated with such deposits must have been activities responsible for initial solution of the iron from soil and rocks and removal by bodies of water. Deposition of iron with rusting of pipes (8, 34, 13) and the fouling of drinking water (4, 8) may become matters of considerable economic importance. In soils, deficiencies of soluble iron may occur under alkaline conditions, excesses under acid conditions, and precipitation with the formation of hardpans in other instances (28, 33).

It has been expressed frequently that iron bacteria are largely responsible for the deposits of bog iron ore which appear in large amounts in many portions of the world. Their relationship, if any, to transformations in soils has received slight attention.

EXPERIMENTAL RESULTS

It is particularly the object of this report to suggest what conditions may favor precipitation and solution as well as oxidation and reduction of iron whether they be created physically or through the action of microörganisms. It appears to be possible to elaborate somewhat on the relationships which microörganisms may have with these transformations in the light of the equations which have been developed in a preceding paper (12).

In the presence of solid ferric hydroxide

I $$\frac{[A_{Fe^{++}}]}{[A_{Fe^{+++}}]} \cdot [A_{H^+}] = \frac{K}{[A_{O_2}]^{1/4}}$$

and II $$\frac{[A_{Fe^{++}}]}{[A_{H^+}]^2} = \frac{K'}{[A_{O_2}]^{1/4}}$$

Under atmospheric conditions creating constant oxygen pressure these may be stated as follows:

III $$\frac{[A_{Fe^{++}}]}{[A_{Fe^{+++}}]} \cdot [A_{H^+}] = K''$$

and IV $$\frac{[A_{Fe^{++}}]}{[A_{H^+}]^2} = K'''$$

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[3] For considerations of iron bacteria and their physiology refer also to the following (4, 6, 7, 8, 9, 20, 22, 24, 25, 39, 40, 42).

These equations show that the ratio of the activities of ferrous and ferric iron, which in dilute solutions will not be appreciably different from the ratio of the concentrations of the inorganic ferrous and ferric iron in solution, will depend upon the reaction and oxygen pressure, two factors which may be altered to a considerable degree by development of microörganisms. Since the reactions of oxidation of iron are reversible, transformations of oxidation may change to reduction depending upon the environmental conditions. In general it may be stated that lowering the oxygen pressure, that is, creating more anaerobic conditions, will: 1, tend to increase ferrous iron at the expense of the ferric, and 2, tend to decrease the hydrogen-ion concentration. Further, increasing the oxygen pressure, such as creating aerobic conditions, will lead toward: 1, a greater amount of ferric iron per unit of ferrous and, 2, a more acid reaction. Increasing the hydrogen-ion concentration with constant oxygen pressure will also tend to diminish the ratio of ferrous iron in solution to dissolved ferric iron by dissolving more of the precipitate at the same time, at equilibrium, holding much more ferrous iron in solution. Lowering the hydrogen-ion concentration with constant oxygen pressure would bring about an oxidation of ferrous iron, with resulting precipitation of the ferric form also increasing the ratio of $\frac{[Fe^{++}]}{[Fe^{+}}$

TABLE 1

Effects of mixed cultures on solution of iron in a dextrose medium

TREATMENT	IRON IN 100 CC. SOLUTION	IRON AS PRECIPITATE IN 100 CC.	TOTAL PER 100 CC.
	mgm.	*mgm.*	*mgm.*
Uninoculated	6	81	87
Inoculated	12	79	91
Uninoculated	0	90	90
Inoculated	10	79	89
Uninoculated	2	88	90
Inoculated	17	73	90
Uninoculated	0	93	93
Inoculated	8	84	92

CONDITIONS PREDISPOSING SOLUTION OF IRON

Under aerobic conditions bacterial cultures should tend to bring iron into solution, provided the reaction became more acid. The following experiment was performed to show this.

Ferrous sulfate was prepared in stock solution, sterilized by passage through a Berkefeld filter and added with aseptic precautions to sterile 100-cc. portions of dextrose medium in 250-cc. Erlenmeyer flasks. This medium contained dextrose, 1 per cent; peptone, 0.01 per cent; NH_4NO_3, 0.05 per cent; and $MgSO_4 \cdot 7H_2O$, 0.01 per cent. The ferrous sulfate was added to make the concentration 0.5 per cent $FeSO_4 \cdot 7H_2O$. The media were buffered with phosphates to create a range of reactions. The phosphates however, precipitated considerable of the iron and made the reaction noticeably acid. Alternate flasks were inoculated with soil and, subsequent to incubation, were analyzed for iron in solution and as precipitate. The ferrous iron in solution was determined by the common method of oxidation with a standard solution of potassium bichromate, potassium ferricyanide being used as an indicator. For total iron the ferric was reduced with stannous chloride and the total iron titrated with bichromate. In some cases aliquots of the solutions were evaporated and ignited, the iron was taken up with HCl, reduced with stannous chloride and titrated with bichromate. Table 1 presents the results of the experiment.

In most instances the iron had been completely precipitated by the phosphates in the medium, but in every instance, as a result of microbial growth under aerobic conditions, iron was brought into solution. This is explained as due to increase in acidity as a result of the decomposition of the dextrose. A contributing factor may have been the slight decrease in oxygen pressure produced as a result of microbial growth, since even under aerobic conditions unless a solution is well aerated there would be a lowered oxygen pressure as a result of the development of the culture.

Under anaerobic conditions it is much easier to demonstrate the solution of iron. Anaerobic conditions in Winogradsky's experiments (39) were used to dissolve ferric iron as a source of soluble ferrous iron for his iron bacteria. Kindler (15) as early as 1836 noted the solution of iron from ferruginous sand about roots. Gruner (10) conducted experiments under anaerobic conditions, which demonstrated solution of iron from rocks and minerals.

It was noted by Lieske (21) that molds exert a reducing action upon ferric compounds. When the mycelial growth was removed from the culture, oxidation occurred. It was believed that reducing enzymes were liberated. It may be, however, that the reduction of the oxygen concentration in the presence of the growing molds might be responsible for the reduction; the oxidation subsequent to removal of the growth might be explained by the action of an increased oxygen concentration. Wright (41) demonstrated that some bacteria exert a solvent action on iron in minerals when these organisms developed on media containing dextrose. It was believed that the extent of solution was due to the hydrogen-ion concentrations developing from the decomposition of dextrose. It is not unlikely that reduced oxygen pressure resulting from development of the organisms exerted some effects.

For the following experiments, freshly precipitated ferric hydroxide was used as the source of iron. To four 1000-cc. flasks considerable amounts of the hydroxide were added. Two flasks remained without the iron. Into 3 flasks, 2 containing ferric hydrate and 1 lacking it, a dextrose solution was introduced. This medium contained dextrose, 1 per cent; $(NH_4)_2SO_4$, 0.01 per cent; K_2HPO_4, 0.01 per cent; $MgSO_4 \cdot 7H_2O$, 0.001 per cent; and $CaCl_2$, 0.001 per cent.

A 1 per cent peptone solution was added to 3 other flasks. All of the solutions were inoculated with soil and introduced well up in the necks of the flasks. The flasks were stoppered with one-holed rubber stoppers carrying tubing which was submerged in water to create rather complete anaerobic conditions. The initial reaction of the media was pH 7.0 and the titer was 0.20 cc. The hydrogen-ion concentration was determined either by means of Clark and Lubs' series of indicators or with a Leeds and Northrup type K potentiometer using a normal calomel half cell as a normal electrode and a Bailey electrode as the hydrogen electrode.

TABLE 2

Effects of anaerobiosis and aerobiosis with mixed cultures upon the solution and precipitation of iron

TREATMENT	pH		TITER*		Fe^{++} IN SOLUTION	
	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic
Dextrose	3.0	3.1	0.90	0.75	0	0
		3.1†		0.75†		0†
Dextrose + Fe(OH)$_3$	6.0	5.3	1.40	0.35	71.88	12.50
		4.2†		0.70†		11.88†
Dextrose + Fe(OH)$_3$	6.0	4.6	1.80	0.90	73.75	21.88
		4.3†		0.90†		12.50†
Peptone	6.2	8.0	0.70	0.00	0	0
		7.8†		0.10†		0†
Peptone + Fe(OH)$_3$	6.4	8.2	1.00	0.00	22.98	0
		8.0†		0.00†		0†
Peptone + Fe(OH)$_3$	6.4	7.7	1.20	0.10	16.25	0
		7.7†		0.00†		0†

* Titer refers to the amount (cc.) of 0.1*N* NaOH which was required to neutralize 5 cc. of the medium, phenolphthalein being used as the indicator.

† Cultures treated with phenol at time of exposure to free air.

Good growth developed in all the solutions. After three weeks the solutions were tested for ferrous iron in solution and for changes in reaction as indicated by pH and titration with standard alkali. The results were presented in table 2.

For the present consider only those results recorded under the headings "anaerobic" which refer to the cultures after anaerobic incubation. As regards the dextrose cultures it is apparent that the hydrogen-ion concentration was much higher in the absence of ferric hydrate even though the titrable acidity was much greater in the presence of the iron. Further, it is apparent that a considerable amount of the iron was brought into solution as a result of bacterial growth. These cultures all gave a very pronounced odor of organic acids, and microscopic observation revealed the development of an abundance of organisms of the *Clostridium pastorianum* type. As stated in the discussion of the equations, the solution of ferric hydrate will tend to occur as a result of lowered oxygen pressure and this reaction will lead to a more alkaline reaction. Under these conditions the microörganisms not only tended to lower the oxygen pressure but also markedly increased the acidity by the decomposition of the dextrose to organic acids. These actions tended to render the reaction extremely acid, as is indicated by the pH of 3.0 in the solution lacking iron. Ferric hydrate, however, prevented the rapid change of pH, which reached a point of only 6.0 in spite of the fact that more acid was actually produced in the presence than in the absence of iron. It is, of course, not alone a solution of the ferric iron but also a reduction to its ferrous form. The solution will hold extremely small amounts of ferric iron under the conditions which developed. It is particularly striking that as much as 70 to 74 mgm. of ferrous iron occurred per 100 cc. of solution at a pH as alkaline as 6.0. As shown in a preceding paper (12), under atmospheric conditions only about 0.00027 p.p.m. of ferrous iron occur in inorganic solution at a pH of 6.0.

Similar though less striking changes occurred with the peptone cultures. The reaction became more acid, as indicated by both the pH and titrable acidity. The initial pH was 7.0 and the titer was 0.20 cc. Although the pH of the culture lacking iron was lower, the titration of the cultures containing iron was greater, showing a greater development of acid and the buffering action exerted by the iron upon the hydrogen-ion concentration as it went into solution. Even with the peptone medium at reactions not far from neutrality, appreciable amounts of iron became dissolved. The increase in acidity may be explained as due to the fatty and carbonic acids formed as a result of the decomposition. It can be stated, therefore, that iron may go into solution under anaerobic conditions not only where compounds low in nitrogen, as carbohydrates, undergo decomposition but also during the decomposition of highly nitrogenous materials. Naturally the more alkaline the reactions the less iron will be retained in solution even under anaerobic conditions, but the amounts will be much greater than could exist in stable form in the presence of high oxygen pressures. Under anaerobic conditions there will also be a relatively high ratio of $\frac{Fe^{++}}{Fe^{+++}}$, so that practically all the iron in solution will occur in the ferrous form. It is further not necessary to postulate the solution as ferrous bicarbonate under such conditions. Any of a great many anions, organic or inorganic, may exist in the solution, with the ions of ferrous iron remaining under anaerobic conditions. There may even be an excess of hydroxyl- over hydrogen-ions under such conditions without eliminating all ferrous iron from solution.

Further studies were made, using a pure culture of *Clostridium sporogenes* and *Escherichia coli*. Media containing peptone or dextrose were prepared as in the preceding experiment. A considerable amount of ferric hydrate was added to each of six 1000-cc. flasks. Dextrose medium was added to three and peptone medium to three. These were sterilized in the autoclave. One of each medium was left sterile and one of each was inoculated with *Cl. sporogenes* and *Esch. coli*. Anaerobic conditions were created by the same means as in the preceding experiment. The results appear in table 3.

The sterilization with heat modified the dextrose medium to the extent of rendering the reaction acid (pH 5.3) and of bringing some of the iron into solution (20.63 mgm. per 100 cc.). It merely brought the peptone solution to a slightly alkaline reaction (pH 7.41).

TABLE 3

Effects of anaerobiosis and aerobiosis with pure cultures upon the solution and precipitation of iron

TREATMENT	DEXTROSE CULTURES				PEPTONE CULTURES			
	pH		Fe^{++} per 100 cc. solution		pH		Fe^{++} per 100 cc. solution	
	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic
			mgm.	*mgm.*			*mgm.*	*mgm.*
Uninoculated	5.34	4.0 4.1*	20.63	19.07 17.51	7.41	8.13 8.03*	0	0 0*
Cl. sporogenes	5.29	4.0 4.1*	34.38	18.75 18.75	6.51	8.30 8.04*	8.73	0 0*
Esch. coli	4.81	4.1 4.2*	66.88	37.19 37.50	6.86	8.16 8.22*	3.75	0 0*

* Cultures treated with phenol at time of exposure to free air.

The data headed "anaerobic" (table 3) shows that much the same changes occurred here as with the mixed cultures. In general the changes are, however, less pronounced. *Cl. sporogenes*, being essentially a proteolytic organism, made limited growth on the dextrose. The reaction became somewhat more acid in both dextrose and peptone media and considerable amounts of iron were dissolved, particularly in the dextrose cultures. Naturally the solution of iron would tend to mask the amounts of acid which were formed and would tend to lower the hydrogen-ion concentration. Iron was dissolved during the development of even these pure cultures under anaerobic conditions. In the cultures of *Esch. coli* in peptone the reaction was very close to neutrality (pH 6.86) but some iron (3.75 mgm. per 100 cc.) was brought into solution.

The preceding discussion has been confined to solution of inorganic compounds of iron. Similar applications of the principles underlying the reactions may be made to compounds of the nature of iron salts of organic acids. The principal factor which will quantitatively modify the transformations of iron will be the ionization of these compounds. In general, ionization of the organic compounds will be lower than that of the inorganic salts of iron. The organic compounds would be considerably more stable than inorganic salts of iron and the differences in stability could be explained as due to the differences in ionization. Those most stable would have very slight ionization, and those of less stability, tending toward precipitation, would have somewhat greater ionization. Conditions, such as decreased hydrogen-ion concentration and increased oxygen pressure, which tend to favor precipitation of inorganic forms of iron may result in no precipitation of organic compounds of the metal.

It may frequently happen that under such conditions as occur in transformations of iron in the presence of organic materials, organic compounds of iron may form which demonstrate much greater stability under aerobic conditions than do inorganic compounds. This is apparently what has taken place in some instances in our experiments where considerable amounts of iron have become oxidized to the ferric form under aerobic conditions and have remained in solution even though the reaction would be such as to hold extremely small amounts of ferric or ferrous ions. To such solutions the additions of considerable amounts of alkali that created very alkaline conditions failed to effect precipitation of iron. One of the compounds which may react in this manner is ferric ammonium citrate.

Lieske (21) observed that sucrose tended to dissolve some iron from hydrated ferric oxide. It was believed that some organic compound of iron was formed. Uspensky (37) found that the addition of small amounts of citrate considerably lowered the concentration of ionized iron. This was explained as being due to the formation of an unionized organic compound of the iron. Gruner (10) likewise found that as a result of the action of peat extracts on iron minerals, products were formed in which the iron was stable in solution or suspension under aerobic conditions for over a year. Bacterial action appeared to be necessary to effect a precipitation of the iron from these solutions unless salts such as $MgCO_3$ were incorporated and the solutions exposed to the air.

With inorganic compounds we may assume that some such reaction as the following takes place as the reaction approaches neutrality:

I $$2Fe^{+++} + nHOH \rightarrow Fe_2O_3 \cdot (H_2O)_{n-3} \downarrow + 6H^+$$

The ferric ions undergo a precipitation as a result of the hydrolysis to a compound which is very slightly soluble under the existing conditions. We may also assume that in the presence of certain organic compounds the following occurs:

II $$Fe^{+++} + H_nR \rightarrow H_{(n-3)}FeR + 3H^+$$

In this case no precipitation will occur, provided that $H_{(n-3)}$ FeR is a compound ionizing to such a slight degree that the solution does not become more than saturated with ferric ions. Since under aerobic conditions extremely few ions remain in solution close to neutrality, precipitation will occur unless the compounds are very slightly ionized. If organic salts of iron are not stable it would be inferred that their ionization liberates more ferric ions than are stable under the existing conditions of hydrogen-ion concentration, oxygen pressure, etc. Such an ionization results in such a reaction as I.

It may happen then, that the iron going into solution under reduced oxygen pressure may become stable under increased oxygen pressure by the formation of organic compounds. In fact, where iron does appear in considerable concentration either in the ferrous or ferric form at reactions close to neutrality it must exist in compounds very slightly ionized and not as ions.

CONDITIONS PREDISPOSING PRECIPITATION OF IRON

The equations presented previously indicate that either increased oxygen pressure or decreased hydrogen-ion concentration, or both, will cause oxidation of iron and may cause its precipitation.

The effects of increased oxygen pressure are shown from a continuation of the studies of cultures initiated under anaerobic conditions and later exposed to the atmosphere. Subsequent to anaerobic incubation, cultures in dextrose and peptone media were divided into 100-cc. portions. Half of the cultures received phenol to inhibit microbial development. The other half were left to permit whatever changes the organisms might produce. Previous to anaerobic incubation the media had been inoculated with soil and no precautions were observed to prevent additional contamination at the time of exposure to the aerobic environment. After two weeks these cultures were analyzed for the remaining ferrous iron and the reaction. The data are included in table 2 under the headings of "aerobic."

It will be noted that as a result of increasing the oxygen pressure there were very slight changes in either the dextrose or peptone cultures which lacked iron initially, except that the reaction became somewhat more alkaline, particularly in the peptone culture. Neither of these cultures, of course, showed iron either subsequent to anaerobic or aerobic incubation. All of the other cultures showed very pronounced disappearance of ferrous iron; in fact, precipitation of ferric hydrate became apparent very soon after exposure of the cultures to the air. At the end of the two weeks there still remained 12 to 22 mgm. of ferrous iron per 100 cc. of solution, but from $\frac{3}{4}$ to $\frac{5}{6}$ of the iron had become oxidized and, to judge from the appearance of the cultures, the ferric iron had become precipitated to a large extent. At the same time the reaction became less acid as indicated by the titration but had higher hydrogen-ion concentrations due to the removal by precipitation of a large part of the iron which had exerted a buffering action. All of the iron became precipitated from the peptone cultures and the reaction became quite alkaline (pH 7.7 to 8.2).

Oxidation and precipitation in these solutions were apparently independent of microbial activity, since the solutions treated with phenol showed practically the same transformations as those not sterilized. The only difference that appears appreciable is that in the dextrose cultures in which somewhat more ferrous iron remained in solution where no phenol was added. It is quite likely that this was due to the depression of the oxygen concentration as a result of the continuation of microbial activity. No such condition developed in the presence of phenol. Such precipitation need not depend upon the presence of catalytic agents as suggested by Cholodny (5). Meehan and Baas-Becking (23) likewise failed to notice that iron bacteria accelerated the oxidation process. At least it is unnecessary to postulate biological action to obtain a speedy transformation.

Similar experiments were performed with the dextrose and peptone solutions, some of which were inoculated with *Cl. sporogenes* and *Esch. coli*. Subsequent to anaerobic incubation these solutions were introduced in 100-cc. portions into 250-cc. Erlenmeyer flasks and exposed to aerobic conditions. After two weeks these were analyzed for ferrous iron and reaction. The results are included in table 3.

Similar changes took place as with the solutions where mixed cultures developed. All of the iron became oxidized and precipitated from the peptone solutions and the reaction became quite alkaline. With the dextrose cultures there was an oxidation of a large part of the iron with an increase in acidity. There appeared to be no appreciable difference between those solutions treated with phenol and those left untreated. Consequently the transformations can be considered as occurring independently of microbial activity. One striking difference between the changes in these solutions and those supporting growth of mixed cultures was the formation of an abundance of precipitated ferric hydrate from the solutions of the mixed cultures and the lack of any visible precipitation from the pure cultures even though the ferrous iron had been oxidized to a considerable degree. As previously stated, such compounds of ferric iron must have formed as would be extremely slightly ionized, in order that no precipitation should occur. It is most likely that these compounds were organic.

It seems particularly striking that even though the reaction may become more acid upon exposure of solutions to aerobic conditions, precipitation takes place. It is frequently inferred that many natural waters, particularly subterranean streams, carry iron in solution as ferrous bicarbonate (8, 13, 40, 10, 5). It is further stated that this form of iron may be quite unstable in contact with free air, causing an oxidation with the precipitation of ferric hydrate. Such reactions, independent of microbial activity may be responsible for the deposition of iron.

Reactions associated with precipitation of manganese may be somewhat

TABLE 4

Transformations resulting from microbial activity in peptone solutions containing iron as ferrous sulfate

TREATMENT	pH		NH_3-N PER 100 CC.		IRON IN SOLUTION				
	Initial	Final	Per culture	Increase due to growth	Total in solution	Total as precipitate	Increase in precipitate due to growth	Total in cultures	Proportion in solution
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
Unfiltered cultures									
Uninoculated.....	5.0	3.6	12.56		141.0	45.5		186.5	75.6
Inoculated........	5.0	4.6	44.00	31.44	129.0	48.5	3.0	177.5	72.7
Uninoculated.....	5.5	3.8	13.68		133.0	53.0		186.0	71.5
Inoculated........	5.5	4.6	44.00	30.32	127.0	57.5	4.5	184.5	68.9
Uninoculated.....	5.9	3.6	4.56		116.0	73.0		189.0	61.4
Inoculated.......	5.9	4.6	16.00	11.44	112.0	70.5	−2.5	182.5	61.4
Uninoculated.....	6.2	3.6	3.44		110.0	76.0		186.0	59.1
Inoculated.......	6.2	5.0	42.24	38.80	66.0	116.0	40.0	182.0	36.3
Uninoculated.....	6.3	3.8	3.44		105.0	81.5		186.5	56.3
Inoculated.......	6.3	5.2	111.92	108.48	36.0	149.0	67.5	185.0	19.5
Uninoculated.....	6.5	3.8	1.12		95.0	91.0		186.0	51.1
Inoculated.......	6.5	4.8	79.36	77.04	70.0	114.5	23.5	184.5	37.9
Uninoculated.....	6.55	4.0	2.32		94.0	94.5		188.5	50.0
Inoculated.......	6.55	5.3	144.48	142.16	6.0	177.5	83.0	183.5	3.3
Uninoculated.....	6.6	3.9	1.12		87.0	100.5		187.5	46.4
Inoculated.......	6.6	5.0	129.12	128.00	42.0	138.5	38.0	180.5	23.3
Uninoculated.....	6.65	4.0	2.32		83.0	107.0		190.0	43.7
Inoculated.......	6.65	5.0	129.12	126.80	0	182.5	75.5	182.5	0
Uninoculated.....	6.7	4.0	1.12		78.0	106.5		184.5	42.3
Inoculated........	6.7	5.3	174.80	173.68	8.0	175.5	69.0	183.5	4.4
Uninoculated.....	6.9	4 2	1.12		77.0	112.0		189.0	40.7
Inoculated.......	6.9	5.5	167.92	166.80	24.0	160.0	48.0	184.0	13.0
Filtered cultures									
Uninoculated.....	5.0	3.6	10.88		134.0	43.0		177.0	75.7
Inoculated.......	5.0	4.5	24.56	13.68	162.0	20.5	−22.5	182.5	88.8
Uninoculated.....	5.5	3.8	12.56		135.5	50.5		186.0	71.5
Inoculated.......	5.5	4.5	22.88	10.32	148.0	29.0	−21.5	177.0	83.6
Uninoculated.....	5.9	3.6	4.56		110.0	58.0		168.0	65.5
Inoculated.......	5.9	5.2	30.88	26.32	88.0	81.5	23.5	169.5	51.9
Uninoculated.....	6.2	3.6	5.68		106.0	61.5		167.5	63.3
Inoculated.......	6.2	4.8	20.56	14.88	100.0	71.5	10.0	171.5	58.3
Uninoculated.....	6.3	3.8	5.12		104.0	59.0		163.0	63.8
Inoculated.......	6.3	5.4	84.00	78.88	36.0	122.0	63.0	158.0	22.8
Uninoculated.....	6.5	4.0	2.32		90.0	60.0		150.0	60.0
Inoculated.......	6.5	5.4	131.36	129.04	14.0	133.0	73.5	147.0	9.5
Uninoculated.....	6.55	3.9	1.12		91.0	54.0		145.0	62.8
Inoculated.......	6.55	5.6	138.24	137.12	10.0	135.5	81.5	145.5	6.9
Uninoculated.....	6.6	4.0	1.12		80.0	33.0		113.0	70.8

TABLE 4—*Continued*

TREATMENT	pH		NH_3-N PER 100 CC.		IRON IN SOLUTION				
	Initial	Final	Per culture	Increase due to growth	Total in solution	Total as precipitate	Increase in precipitate due to growth	Total in cultures	Proportion in solution
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
Filtered cultures—Continued									
Inoculated.......	6.6	5.8	140.48	139.36	2.0	114.5	81.5	116.5	1.7
Uninoculated.....	66.5	4.0	1.12		65.0	35.5		100.5	64.7
Inoculated.......	6.65	5.4	86.80	85.68	0	103.0	67.5	103.0	0
Uninoculated.....	6.7	4.1	1.12		58.0	31.0		89.0	65.2
Inoculated.......	6.7	4.5	44.56	43.44	12.0	78.0	47.0	90.0	13.3
Uninoculated.....	6.9	4.25	1.12		58.0	28.5		86.5	67.1
Inoculated.......	6.9	6.0	115.36	114.24	0	78.0	49.5	78.0	0

different from those related to precipitation of iron. Söhngen (35) observed that the oxidation of manganous compounds, resulting in the precipitation of manganic oxide, was related to the presence of oxy-acids. In the presence of certain hydroxyl-ion concentrations the oxidation and precipitation took place. This occurred whether the oxy-acids were produced by microörganisms or incorporated directly in the solutions and treated with sodium bicarbonate. Fatty acids failed to effect the change. This reaction is particularly interesting in that oxidation of manganous compounds with precipitation of manganic oxide occurs chemically with much less speed than oxidation and precipitation of iron. It is also significant that reduction of manganic oxide took place by the action of microörganisms in the case where oxy-acids were produced. This reduction did not occur in the presence of fatty acids or inorganic acids, with the exception of HCl. These results may throw added light on the precipitation of manganese observed by Beijerinck (3) and Thiel (36).

There is another phase of the phenomenon of precipitation of inorganic compounds of iron under aerobic conditions. In certain cases iron introduced as inorganic compounds into solutions containing organic matter may become precipitated subsequent to development of microörganisms. The following experiments were performed to indicate some of the agencies which under aerobic conditions might lead to precipitation of iron introduced as ferrous sulfate into peptone solutions. A 1 per cent solution of peptone was used. To portions of the solution, graduated amounts of NaOH were added to create differences in reaction. For each of two series 22 Erlenmeyer flasks of 250 cc. capacity were used and 100 cc. of the medium was introduced into each. For one series ferrous sulfate was added to solution sufficient for 22 flasks. The final concentration of $FeSO_4 \cdot 7H_2O$ was 0.5 per cent. The entire solution was sterilized by passage through a Berkefeld filter and then introduced with aseptic precautions into the sterile Erlenmeyer flasks in 100-cc portions. To these media were added different amounts of 0.2 *N* NaOH to create a range of reactions. One flask of each reaction was left uninoculated as a control and one of each was inoculated with a soil suspension.

Another large volume of the peptone-ferrous sulfate solution was divided into 250-cc. portions. The reaction of each of these portions was adjusted with amounts of alkali to create a series corresponding to that of the first series, but the large amount of precipitate which formed as a result of the alkali was filtered off and each lot of solution sterilized separately by passage

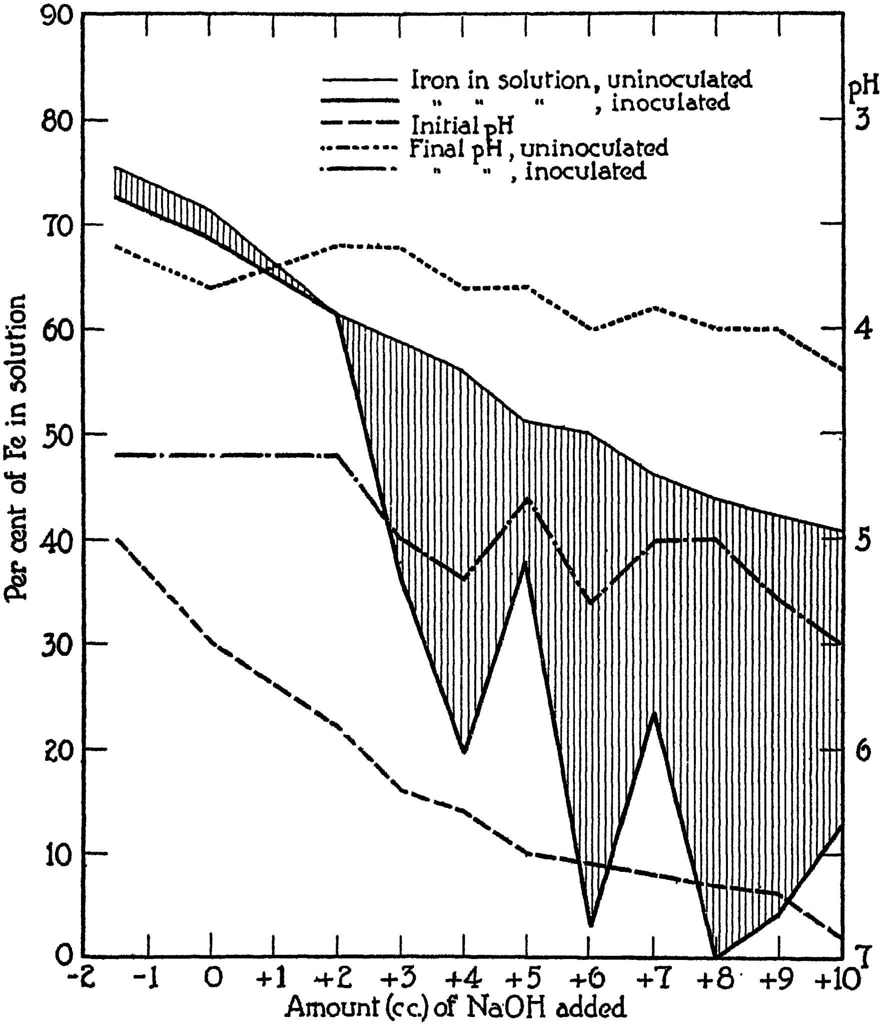

FIG. 1. CORRELATION BETWEEN CHANGES IN REACTION AND PRECIPITATION OF IRON IN UNFILTERED CULTURES

through Berkefeld filters. These solutions were likewise introduced into sterile 250-cc. Erlenmeyer flasks under aseptic precautions in 100-cc. amounts. One flask of each reaction was inoculated with soil and one left uninoculated to act as a control. The solutions were incubated for a month and then

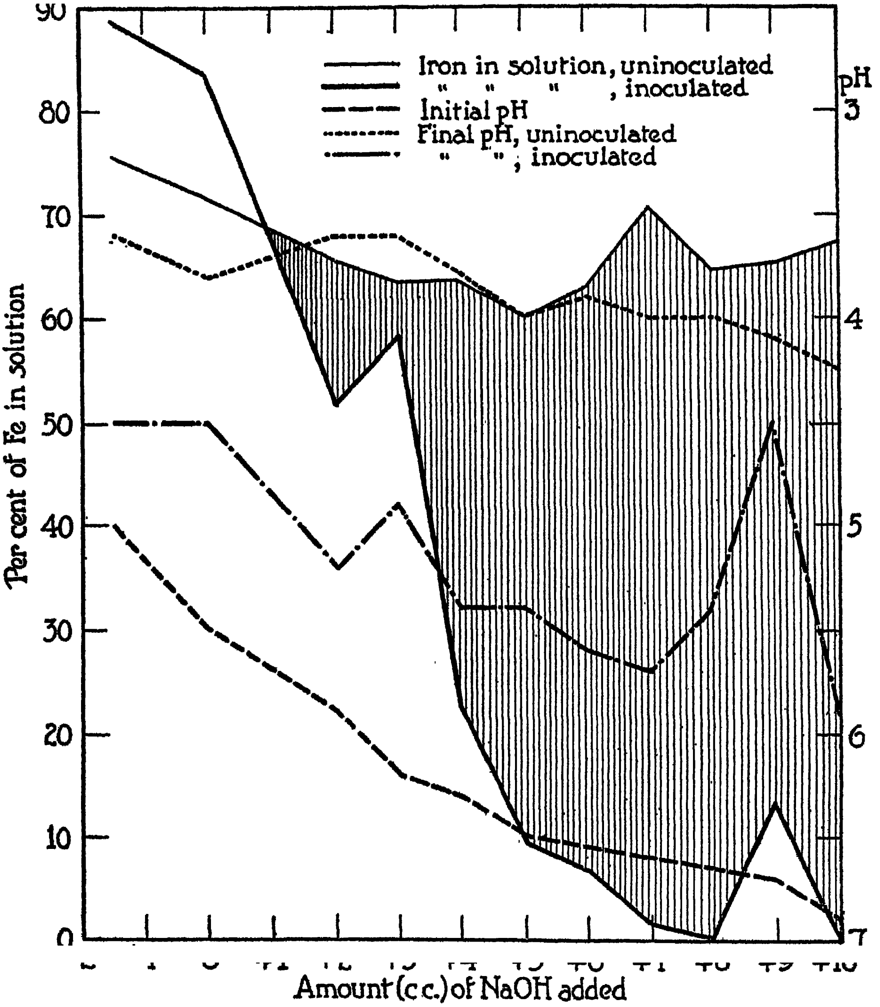

FIG. 2. CORRELATION BETWEEN CHANGES IN REACTION AND PRECIPITATION OF IRON IN FILTERED CULTURES

analyzed for total and ferrous iron in solution, iron as precipitate, ammonia-nitrogen, and pH. The results are presented in table 4 and figures 1, 2 and 3. As these cultures incubated, an abundance of precipitate of ferric hydrate developed in the inoculated solutions. In some cultures this appeared on the surface in the form of heavy membranes and in others it formed as a flocculent precipitate. Even the sterile media which had been filtered at the time of adjusting the reactions with alkali showed an appreciable formation of precipitate. The explanation for the precipitation becomes apparent upon a consideration of the environmental conditions. The solutions were adjusted to create reactions between pH 5.0 and 7.0. At such reactions, however, very small amounts of ionized iron will remain in solution. Consequently the reaction proceeds to oxidation to the ferric form, and becomes hydrated and precipitated as ferric hydrate, at the same time creating a more acid reaction (see discussion p. 382). The oxidation and precipitation will continue until equilibrium is reached at which point the solution becomes saturated with ferric iron and the ratio of $\frac{[Fe^{++}]}{[Fe^{+++}]}$ reaches a definite value determined by the hydrogen-ion concentration under constant oxygen pressure. Where fairly large amounts of iron are introduced, as under the conditions of this experiment, there will be considerable precipitation until the acidity is noticeably great before equilibrium is reached. In these experiments the control cultures which were initially adjusted to reactions from pH 5.0 to 7.0, dropped to pH 3.6 to 4.25 at equilibrium. This precipitation continued for some time after the media were prepared, consequently there was a fairly large accumulation of ferric hydrate even in the media filtered at the time of preparation. The relative extent of the changes in reaction is shown in figures 1 and 2.

Although the reactions became noticeably acid as a result of the precipitation, considerable growth of bacteria developed except in the most acid cultures. Even here some growth occurred. As a result of microbial growth the reaction became less acid in all instances but was most marked where bacterial growth was greatest. The pH of the cultures, which, according to the controls, was 3.6 to 4.25, reached points between 4.6 and 6.0, because of microbial development. Increases in pH are correlated with increases in precipitation of iron and, in general, the greater the reaction change the less the iron which remained in solution. In figures 1 and 2 the areas above the lines of the shaded areas indicate the percentages of iron in the cultures occurring as precipitate and below these, the percentages of iron in solution. The areas above the heavy lines at the lower parts of the shaded areas indicate the percentages of iron as precipitate in corresponding cultures. The areas below these lines indicate the percentages of iron remaining in solution even after microbial development. Consequently the shaded areas between the two lines indicate the amounts of iron precipitated as a result of bacterial growth in terms of percentages of the total iron contained in the cultures.

It is evident that large amounts of iron became precipitated except in the cultures which were most acid initially. In general, there is also a correlation between the amount of precipitation and the extent of the change in reaction toward alkalinity.

The precipitation of iron tends to increase the acidity. Further, the development of microörganisms tends to lower the oxygen pressure, which would favor solution rather than precipitation of iron. The factor responsible for precipitation must then be one largely concerned with formation of alkali. The alkali formed not only neutralized the acid liberated by precipitation of the iron but also further decreased the hydrogen-ion concentration to such an extent as to more than offset the dissolving action of the reduced oxygen pressure.

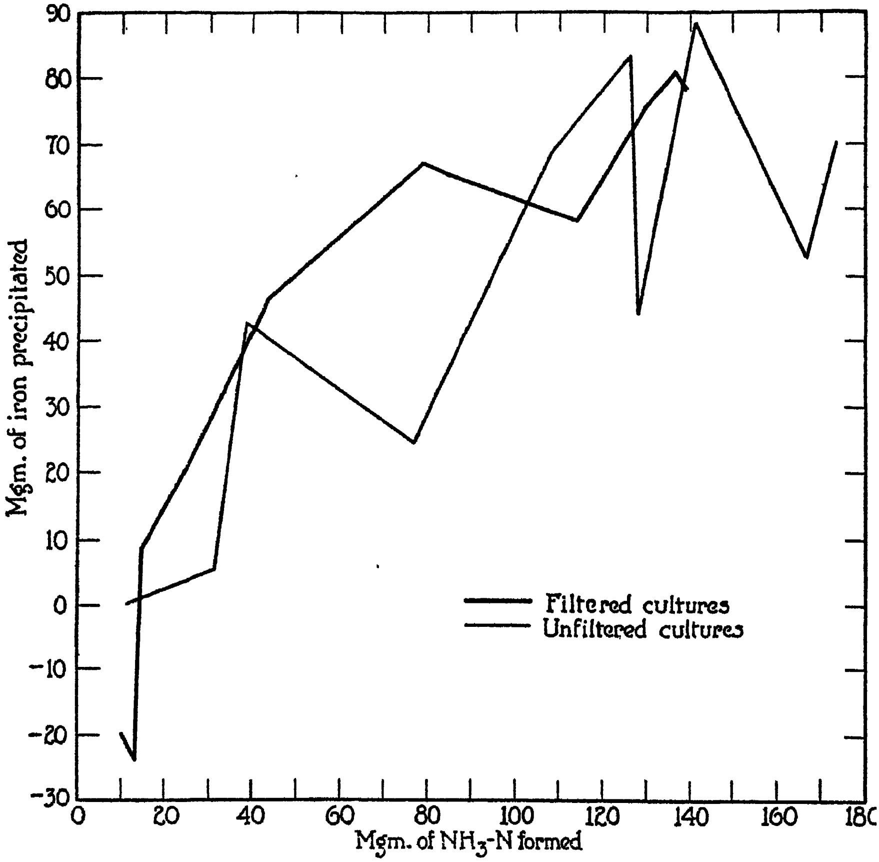

Fig. 3. Correlation Between Formation of Ammonia and Precipitation of Iron as a Result of Development of Bacteria in Peptone Solutions

In figure 2 the most acid cultures showed a greater solution of iron than the uninoculated ones even though the acidity was less than in the uninoculated. This may be explained as due to the effects of microbial growth in lowering the oxygen pressure.

Since these media contained peptone, the decrease in acidity was probably caused by the formation of ammonia as a result of microbial development. It is not to be expected that there would be an absolute correlation between ammonia formation and precipitation, since other factors, such as the effect of lowered oxygen pressure as a result of development of bacteria, would tend to offset the action of ammonia formation. However, from the curves in figure 3 it is quite evident that the extent of the formation of ammonia is sufficiently correlated with precipitation of iron to ascribe to it major importance in determining the precipitation of ferric hydrate. In general, the greater the amount of ammonia formed the greater the amount of iron precipitated. The changes produced under these experimental conditions could hardly be considered as typifying conditions under which the reactions proceed in nature although reactions of this sort do undoubtedly contribute to natural transformations. They may, however, explain results obtained by others under similar conditions and invalidate conclusions that such precipitation is in any way associated with iron bacteria or that iron is directly concerned with the vitality of the cells responsible for the precipitation. The organisms provoke the precipitation but do so only indirectly, and any organism capable of producing ammonia in abundance from organic nitrogenous compounds under aerobic conditions would undoubtedly create similar precipitation.

Precipitation may also occur under anaerobic conditions. In solutions containing iron, wherever sulfides are formed either from organic compounds or as a result of reduction of inorganic compounds of sulfur one may observe the formation of iron sulfide (10, 13). This is because of extremely low solubility product of the compound, which results in precipitation under conditions that would permit solution of ferric hydrates. The precipitation of iron under such conditions has been used as an index of sulfide formation by anaerobes (11, 14) and the occurrence of anaerobes in water as an index of contamination (38).

The discussion above has been concerned principally with precipitation of inorganic forms of iron from solutions. Of no less interest is the precipitation of iron from organic compounds of such a nature as ferric ammonium citrate early noted by Adler (1) and studied more recently by Lieske (21, 22), Harder (13), Mumford (31), Thiel (36), and Mudge (29, 30). Although believed by Molisch (24, 25, 27) to explain the mechanism of precipitation by iron bacteria, this has been shown to be a phenomenon entirely divorced from transformations associated with the nutrition of such organisms. In fact it appears that such changes are chemically and biologically of little more significance than the decomposition of specific organic materials. As has already been stated, iron remains in solution as organic compounds under aerobic conditions close to neutrality, by reason of the low ionization of these compounds. Transformation to compounds of greater ionization will result in precipitation of iron at reactions close to neutrality. This is undoubtedly the result of the decomposition of the organic radicals to which the iron is bound, liberating the iron into more ionized form. Hydrolysis and precipitation naturally follow. It would appear that almost any organism having the capacity of decomposing these organic radicals would effect the precipitation of the iron. It does not appear to be particularly striking that organisms of a great variety having the capacity of precipitating iron from organic compounds may be found widely distributed in nature. It would seem to be more unusual if they were not numerous. As regards such precipitation, it becomes apparent that the specificity is not for the iron but rather for the organic radical. The fact that the specificity of such a precipitation is associated with the decomposition of the organic radical is clearly demonstrated by the results of Murray and Skinner (32). They made use of such precipitation for the differentiation of coli-aerogenes types which are not readily distinguished from one another. As shown by Koser (16, 17, 18, 19) these forms may be differentiated on the basis of utilization of citrate. For a convenient method of performing the test, Murray and Skinner utilized solid media containing ferric ammonium citrate. Those forms utilizing the citrate caused precipitation of iron with the formation of brown zones about the colonies whereas those not decomposing the citrate formed no brown zones. Although precipitation by some such means may contribute to natural precipitation of iron, one would hardly be justified in assuming that the iron played any part of primary importance in the nutrition of the cells causing the deposition.

OXIDATION AND REDUCTION OF IRON

The conditions favoring oxidation of iron have been stated as being either an increase in the oxygen pressure, a decrease in the hydrogen-ion concentration, or both. Such changes may or may not result in precipitation, depending upon the extent of the ionization of the iron. In solutions which are saturated with respect to iron, precipitation will occur subsequent to oxidation unless extremely few ions of iron exist. Where organic forms of iron do occur, oxidation may take place without precipitation. Wherever inorganic forms of iron do occur and even with some of the more highly ionized organic compounds, precipitation will occur subsequent to oxidation. It can hardly be stated, therefore, that oxidation results invariably in precipitation of iron. Conversely, precipitation may occur independently of oxidation of iron. Under such conditions as have been discussed previously, precipitation may result subsequent to the decomposition of organic radicals with which iron is in combination. Whether the iron in solution occurs in organic un-ionized or inorganic ionized forms, it will tend to become oxidized by increasing the oxygen pressure or lowering the hydrogen-ion concentrations. In the presence of either slightly ionized or strongly ionized compounds the ratio of $\frac{[Fe^{++}]}{[Fe^{+++}]}$ will be the same at constant conditions of oxygen pressure and reaction except for whatever influences other ions in solution may exert.

DISCUSSION

Some of the agencies which may be responsible for precipitation of iron under natural conditions are the following:

1. Activity of iron bacteria, both the strict and facultative autotrophs.
2. Activity of heterotrophic microörganisms in decomposing organic compounds of iron.
3. Activities of organisms causing precipitation of iron as a result of environmental changes, such as the formation of alkali (NH_3) or the increase in oxygen pressure (algae).
4. Strictly chemical changes occurring as results of environmental changes, such as increase in oxygen pressure.

Precipitation from any of the first three of these agencies is the direct or indirect result of microbial activity. In all four cases the iron may have become dissolved initially through the indirect action of microörganisms under conditions of reduced oxygen pressure. Solution may also be quite independent of microörganisms in an environment conducive to reduction. Which of these may have been of major importance in the deposition of bog iron ore is quite uncertain. Activities other than the above may have been active. It would appear to be assuming too much to state that iron bacteria have been mainly responsible for the formation of these deposits, merely because they are generally found in regions of iron precipitation.

Supposing they are active in the formation of the natural deposits we must assume that they can develop where chemical precipitation would not proceed, or assume that they accelerate the change which might occur spontaneously. This latter assumption appears the more logical. As far as is known, all iron bacteria are aerobic. In fact, the reaction by which they exist is one of oxidation, and one of the characteristic changes always associated with the activity of iron bacteria is the accumulation of precipitated ferric hydrate about the cells. Also the reaction by which they exist is reversible. Let us assume a condition of equilibrium as regards ferrous and ferric iron in solution. If iron bacteria should become active here the concentration of both ferrous iron and oxygen would become reduced and ferric hydrate would be precipitated. The solution would consequently be in a state conducive to reduction and the precipitated iron would again go into solution. Unless the solution is not in equilibrium but in a state conducive to oxidation, accumulation of a precipitate of ferric hydrate cannot occur. It would appear then that iron bacteria exist upon a very narrow margin, utilizing energy from a reaction which is occurring spontaneously at no negligible speed. Assumptions by Winogradsky (39, 40) and Lieske (20) that the reaction takes place under conditions where it would not proceed independently of the bacteria, need further experimental verification.

The reactions by which iron bacteria exist are quite different from those utilized by sulfur bacteria. The oxidation and reduction of iron is a reversible reaction and may occur spontaneously, depending upon no other changes than variations in reaction or in oxygen pressure. With changes in sulfur, however, the transformations from H_2S to SO_4^{--} and the reverse do not take place by the mere effects of hydrogen- or hydroxyl-ions or of variations in oxygen pressure, at least to a rate which is readily detected. Consequently with sulfur there must be more specific oxidizing or reducing agents than may effect iron transformations.

The precipitation of iron about algae, although possibly of minor importance in the accumulation of deposits of bog ore (5), appears to be of rather general occurrence (5, 25, 26, 27). This reaction may be associated indirectly with the photosynthetic activity of the plants whereby oxygen pressure becomes greater in the immediate vicinity of the developing plants.

From the preceding considerations it should become apparent that biologically the mere precipitation of iron is of little significance unless the reactions responsible for the change are understood. It also seems likely that iron precipitation is much less frequently the result of action of iron bacteria than is generally expressed. The recent observations, both theoretical and practical, by Baas-Becking and coworkers (2, 23) seriously question even the existence of any organisms which live by a reaction ascribed to iron bacteria.

It is realized by the authors that the effects of many compounds or organic or inorganic ions which may occur in solutions where transformations of iron take place, may affect not only the speeds of the changes but also quantitatively influence the solution of iron. How great their effects may be is undetermined, but it would not appear that they would modify essentially the reactions considered above.

SUMMARY

Some of the activities of microörganisms associated with solution, precipitation, oxidation, and reduction of iron are considered in the light of equations developed previously. The following observations were noted:

1. Under aerobic conditions microörganisms may effect solution of iron as a result of formation of acid by development in dextrose solutions.
2. Under anaerobic conditions in dextrose or peptone media, microörganisms may dissolve and reduce iron present as ferric hydrate as a result of decreasing the oxygen pressure and of the formation of acid. This may occur even at reactions close to neutrality.
3. Iron may remain in solution in organic combination under conditions inhibitory to the solution of inorganic iron, because of the extremely low ionization of the iron in organic compounds.
4. Organic compounds of iron may form subsequent to solution of iron in organic media.
5. Upon atmospheric exposure of solutions containing iron dissolved and reduced under anaerobic conditions, oxidation takes place and precipitation may occur. These changes may be independent of microbial activity.
6. Under aerobic conditions, iron as ferrous sulfate introduced into peptone solutions may become precipitated subsequent to microbial development. The amount of precipitation is correlated with the amount of ammonia formed as a result of the decomposition of the peptone.
7. The precipitation of iron from organic compounds of the metal results from the decomposition of the organic radicals creating a greater abundance of iron ions than existed in the original solution and more such ions than would create a saturation. Such precipitation

is dependent upon the activity of organisms capable of decomposing the organic radicals and not upon the direct action of the microörganisms upon the iron.

8. Oxidation of iron may or may not result in precipitation of ferric iron. Precipitation of ferric iron does not necessarily indicate an immediately preceding oxidation.

9. So many agencies are active in the precipitation of iron that biologically it appears to be of little significance unless the reactions responsible for the change are understood.

REFERENCES

(1) Adler, O. 1904 Ueber Eisenbakterien in ihrer Beziehung zu den therapeutisch verwendeten natürlichen Eisenwassern. *Centbl. Bakt.* (II) 11: 215–219, 277–287.

(2) Baas-Becking, L. G. M., and Parks, G. S. 1927 Energy relations in the metabolism of autotrophic bacteria. *Physiol. Rev.*, 7: 85–106.

(3) Beijerinck, M. W. 1913 Oxydation des Mangancarbonates durch Bakterien und Schimmelpilze. *Folia Microbiol.* 2: 123–134.

(4) Brown, J. C. 1904 Deposits on pipes and other channels conveying potable water. *Proc. Inst. Civ. Engin.* 156: (part 2) 1–17. [Cf. Harder (12).]

(5) Cholodny, N. 1926 Die Eisenbakterien, p. 1–162. Jena.

(6) Cohn, F. J. 1875 Ueber den Brunnenfaden (Crenothrix polyspora) mit Bemerkungen über die mikroskopische Analyse des Brunnenwassers. *Beitr. Biol. Pflanz.* 1: 108–131.

(7) Ehrenberg, C. G. 1836 Vorläufige Mittheilungen über das wirkliche Vorkommen fossiler Infusorien und ihre grosse Verbreitung. *Poggendorff's. Ann. Phys. u. Chem.* 38: 213–227.

(8) Ellis, D. 1919 Iron bacteria: 1–179. London.

(9) Gicklhorn, J. 1920 Studien an Eisenorganismen. I. Mitt. Über die Art der Eisenspeicherung bei Trachelomonas und Eisenbakterien. Sitzungsberichte Kaiserliche Akad. der Wissenschaften in Wien. Math. Naturwiss. Klasse. Abt. I, 129: 187–213.

(10) Gruner, J. W. 1922 The origin of sedimentary iron formations: The Biwabik formation of the Mesabi range. *Econ. Geol.* 17: 407–460.

(11) Hall, I. C., and Peterson, E. 1924 The discoloration of brain medium by anaerobic bacteria. *Jour. Bact.* 9: 211–224.

(12) Halvorson, H. O., and R. L. Starkey 1927 Studies on the transformations of iron in nature. I. Theoretical considerations. *Jour. Phys. Chem.* 31: 626–631.

(13) Harder, E. C. 1919 Iron depositing bacteria and their geologic relations. *U. S. Geol. Survey, Prof. Paper* 113: 1–89.

(14) Kahn, M. C. 1925 Hydrogen sulfide production by anaerobic spore forming bacteria. *Jour. Bact.* 10: 439–447.

(15) Kindler, A. 1836 Bemerkungen über die Bildung einiger Eisenerze. *Poggendorff's Ann. Phys. u. Chem.* 37: 203–206.

(16) Koser, S. A. 1923 Utilization of the salts of organic acids by the coli-aerogenes group. *Jour. Bact.* 8: 493–520.

(17) Koser, S. A. 1924 Correlation of citrate utilization by members of the coli-aerogenes group with other differential characteristics and their habitat. *Jour. Bact.* 9: 59–77.

(18) Koser, S. A. 1924 Is ability to utilize citrate readily acquired or lost by the colon-aerogenes group? *Jour. Infect. Diseases* 35: 315–322.

(19) Koser, S. A. 1926 Differential tests and their relation to habitat of the coli-aerogenes group. *Abs. in Jour. Bact.* 11: 77–78.

(20) Lieske, R. 1911 Beiträge zur Kenntnis der Physiologie von Spirophyllum ferrugineum Ellis, einem typischen Eizenbakterien. *Jahrb. Wiss. Bot.* 49: 91–127.

(21) LIESKE, R. 1912 Untersuchungen über die Physiologie eisenspeichernder Hyphomyceten. *Jahrb. Wiss. Bot.* 50: 328–354.
(22) LIESKE, R. 1919 Zur Ernährungsphysiologie der Eisenbakterien. *Centbl. Bakt.* (II) 49: 413–424.
(23) MEEHAN, W. J., AND BAAS-BECKING, L. G. M. 1927 Iron organisms. *Science* (n. s.) 66: 42.
(24) MOLISCH, H. 1892 Die Pflanze in ihren Beziehungen zum Eisen. Jena [Cf. Molisch (25)].
(25) MOLISCH, H. 1910 Die Eisenbakterien, 1–83. Jena.
(26) MOLISCH, H. 1910 Über die Fällung des Eisens durch das Licht und grüne Wasserpflanzen. *Sitzber. Akad. Wiss. Wien. Math. Naturw. Kl.* 119 (Abt. I): 959–984.
(27) MOLISCH, H. 1926 Pflanzenbiologie in Japan und Grund eigener Beobachtungen. Jena [Cf. *Centbl. Bakt.* (II) 68: 359–365].
(28) MORISON, C. G. T., AND SOTHERS, D. B. 1914 The solution and precipitation of iron in the formation of iron pan. *Jour. Agr. Sci.* 6: 84–96.
(29) MUDGE, C. S. 1926 The presence of iron depositing bacteria in milk. *Proc. Soc. Exp. Biol. and Med.* 23: 725–726.
(30) MUDGE, C. S. 1926 The possible rôle of iron depositing bacteria in the formation of hardpan. *Proc. Soc. Exp. Biol. and Med.* 23: 726–727.
(31) MUMFORD, E. M. 1913 A new iron bacterium. *Chem. Soc. Jour. (London)* 103: 645–650.
(32) MURRAY, T. J., AND SKINNER, C. E. 1925 Differentiation of B. aerogenes and B. coli of non-fecal origin from B. coli of fecal origin. *Proc. Soc. Exp. Biol. and Med.* 23: 104–106.
(33) ROTHER, G. 1912 Über die Bewegung des Kalkes, des Eisens, der Tonerde und der Phosphorsaure und die Bildung des Ton-Eisenortsteins im Sandboden. Dis. Berlin 1912. (Abs. in *Neues. Jahrb. Min. Geol. und Paläon.* (1913) 2: 412.)
(34) SCHORLER, B. 1904 Beiträge zur Kenntnis der Eisenbakterien. *Centbl. Bakt.* (II) 12: 681–695. Die Rostbildungen in den Wasserleitungsröhren. 1906. *Ibid.* 15: 564–568.
(35) SÖHNGEN, N. L. 1914 Umwandlungen von Manganverbindungen unter dem Einfluss mikrobiologischer Prozesse. *Centbl. Bakt.* (II) 40: 545–554.
(36) THIEL, G. A. 1925 Manganese precipitated by microörganisms. *Econ. Geol.* 20: 301–310.
(37) USPENSKY, E. E. 1924 Contributions to the study of the action of different quantities of iron. Solution acting as buffer towards iron. *Trans. Inst. Fert. (Russia)* 23: 1–32.
(38) WILSON, W. J., AND BLAIR, E. M. McV. 1925 Correlation of the sulfite reduction test with other tests in the bacteriological examination of water. *Jour. Hyg.* 24: 111–119.
(39) WINOGRADSKY, S. 1888 Ueber Eisenbakterien. *Bot. Ztg.* 46: 262–270.
(40) WINOGRADSKY, S. 1922 Eisenbakterien als Anorgoxydanten. *Centbl. Bakt.* (II) 57: 1–21.
(41) WRIGHT, D., JR. 1922 Equilibrium studies with certain acids and minerals and their probable relation to the decomposition of minerals by bacteria. *Calif. Univ. Pubs. Agr. Sci.* 4: 245–337.
(42) ZOPF, W. 1882 Zur Morphologie der Spaltpflanzen. Leipzig. [Cf. Cholodny (5).]

A STUDY OF BASE EXCHANGE IN SOILS WITH THE AID OF THE QUINHYDRONE ELECTRODE

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Formulas are developed with the aid of the Donnan membrane equilibrium principle for base exchange in soils. They are verified by titrating suspensions of clay acid with salt solutions in the presence of a quinhydrone electrode. The results are then used to evaluate the base-exchange constants for several pairs of cations, the ionization constants of the clay acid and several clay salts, and the combining or molecular weight of the solid matter in the clay suspension.

The subject of zeolitic base exchange in soils has deservedly attracted much attention from soil scientists (4, 5, 6, 9, 10); and with permutit, a zeolite used for water softening, much important work has been done, including that of Rothmund and Kornfeld (7, 8, 11, 12), who have worked out an empirical formula,

$$\left(\frac{x}{n-x}\right)^p \cdot \frac{b-x}{a-x} = K$$

where p represents the amount of exchangeable base in the permutit expressed in terms of an equivalent, a and b are the equivalents of each cation present in the solution, and p and K are empirical constants which depend upon the nature of the exchange bases. Their investigations prove that a true equilibrium is reached very quickly from either side, although possibly complicated by the hydrolysis of the permutit in some cases. A new derivation of this formula is given in the following.

For most of this investigation a clay acid was used which had been practically freed from cations other than the hydrogen ion. A few experiments have been carried out using clay "salts" prepared by treatment with a large excess of solution containing the cation of the salt desired. The structure of the clay acid has been the subject of many speculations (3), but for our purpose it will be sufficient to give it the formula HC (or H_2C').

On bringing a solution containing a uni-univalent salt, such as potassium chloride, into contact with clay, an equilibrium is quickly established according to the equation $HC + KCl \rightleftharpoons KC + HCl$. Some of the hydrogen of the clay acid and some of the potassium of the clay salt are dissociated from the clay and take part in the formation of the well-known electric double-layer. This thin film around the clay particle will be spoken of as solution I, and the bulk of the solution is solution II. For the two dissociation equilibria the mass action law gives:

$$\frac{[H^+]\,[C^-]}{[HC]} = K_H \tag{A}$$

and

$$\frac{[K^+]\,[C^-]}{[KC]} = K_K \tag{B}$$

Since the clay aggregate fulfils the condition of being non-diffusible, the Donnan membrane equilibrium principle may be applied to solutions I and II, giving:

$$\frac{[H^+]_I}{[K^+]_I} = \frac{[H^+]_{II}}{[K^+]_{II}} \tag{C}$$

Substituting (A) and (B) in (C) and simplyfying,

$$\frac{[H^+]\,[KC]}{[K^+]\,[HC]} = \frac{K_H}{K_K} = Kex \tag{D}$$

where the hydrogen- and potassium-ion concentrations are those in the bulk of the solution. For the reaction of a divalent cation, like calcium on clay acid, the equation becomes:

$$\frac{[H^+]^2\,[CaC]}{[Ca^{++}]\,[HC]^2} = \frac{K_H{}^2}{KCa} = Kex \tag{E}$$

Equations (D) and (E) have been derived by making the assumption that a single hydrogen of each clay "molecule" is replaced. If the clay acid acts as a dibasic acid, another theoretical treatment is necessary. For this purpose, by assuming that all the hydrogens are equidistant from the mean position of the negative charge on the clay particles, the application of Coulomb's law becomes practicable. Let the work required to split off one hydrogen ion be taken as a unit. The work required to split off two hydrogen ions simultaneously will be proportional to the product of the charges, i.e., the two times two, or four units. Therefore, for the second ion, four minus one, or three units would be required. In the same way five units of work would be performed in removing a third hydrogen. According to the free energy equation, $W = RTlnK$, if $W_2 = 3W_1$, if $W_1W_2 = 3W_1$ and if $W_1\,W_2\,W_3 = 9W_4$, then $W_1\,W_2 \ldots Wp = p^2W_1$ and $K_1K_2 = K^4$, $K_1K_2K_3 = K_1^9$ and $K_1K_2 \ldots Kp = K_1{}^{p^2}$, K_1 with the exponent p squared.[1]

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[1] The application of this principle to some of the simpler inorganic polybasic acids is of interest. In some of these, e.g., phosphoric acid, the successive ionization constants derived on this basis seem to lie within the experimental error of the constants given in the literature. In the case of certain polybasic organic acids an application of this principle might lead to an estimation of the spatial arrangement of the replaceable hydrogens (10).

If more than a single hydrogen of the clay acid molecule is replaced, other equations with other constants must be substituted for (A) and (B). If only part of the second hydrogen is replaced, the constant in the equation will be a statistical mean of the two constants, suitably expressed. This could be handled by the method of Rothmund and Kornfeld (8) in which the ratio [KC]/[HC] could be raised to that power which would best fit the results to the interpretation of which the reasoning based on Coulomb's law might be applied. The simple equation (D) and (E) will be first applied to the results and if found inadequate, recourse may then be had to the equation:

$$\frac{[\mathrm{H}^+]^p}{[\mathrm{K}^+]^p}\,\frac{[\mathrm{K}p\mathrm{C}]}{[\mathrm{H}p\mathrm{C}]} = \frac{[\mathrm{H}_{\mathrm{H}_1}]^{p^2}}{[\mathrm{K}_1]^{p^2}} = \mathrm{K}ex \tag{F}$$

or

$$\frac{[\mathrm{H}^+]^{2p}}{[\mathrm{Ca}^{++}]^p}\,\frac{[\mathrm{C}ap\ \mathrm{C}_2]}{[\mathrm{H}p\mathrm{C}]^2} = \frac{[\mathrm{K}_{\mathrm{H}_1}]^{2p^2}}{[\mathrm{Ca}^{++}]^{p^2}} = \mathrm{K}ex \tag{G}$$

During the titration H ions are set free in the main solution, as an equivalent amount of the base ion enters into combination with the clay so that $[\mathrm{H}^+] = [\mathrm{KC}] = x$. Set the $[\mathrm{K}^+]$ in the solution equal to y and the [HC] will equal $(a - x)$ where a is the total number of equivalents of the first replaceable hydrogen per liter. Equation (D) then becomes:

$$\frac{x^2}{(a - x)y} = \mathrm{K}ex \tag{H}$$

and equation (E) for divalent ions:

$$\frac{x^3}{(a - x)^2 y} = \mathrm{K}ex \tag{I}$$

For any highly ionized salt, having a cation of valence n, the general equation

$$\frac{x^{(n+1)}}{(a - x)^n\, y} = \mathrm{K}ex \tag{J}$$

might be obtained in a similar manner, in which n represents the valence of the cation. (In this discussion it is assumed that the anion of the added salt produces no complications.) Similar equations have been derived from (F) and (G).

The x values were obtained by measuring the concentration of the H ion in solution with a quinhydrone electrode and the y values were calculated by subtracting x from the total concentration of the salt in solution. By substituting various pairs of experimental results in (F) and solving simultaneously, a value for a is obtained which, if reasonably constant and if in accord with the known behavior of the various ions involved, may be considered as evidence in support of the above derivations. From this value it will be a simple matter, moreover, to calculate the apparent molecular (or combining) weight of the clay colloid. By setting p in (F) equal to 2 we have an equation available for the case where two adjacent ions, both attached to the same clay molecules, have been simultaneously replaced, according to the equation:

$$H_2C' + 2M^+ \leftrightarrows M_2C' + 2H^+ \quad \text{or} \quad H_2C' + M^{++} \leftrightarrows MC' + 2H^+$$

EXPERIMENTAL

The salt solutions used for these experiments were made from Baker's analysed chemicals and freshly boiled distilled water. All of the glassware was Pyrex and was carefully cleaned with chromic acid solution before use. The standard cell and potentiometer were carefully calibrated before and after use.

Preparation of acid clay

About 200 ml. of a suspension of Putman Clay[2] was placed in a parchment container of the dimensions 1 x 7 x 12 cm. and dialyzed for two weeks with the aid of direct current at 110 volts passing between platinum electrodes. The amperage was at first 80 milliamperes, but toward the end of the experiment it dropped to 12 milliamperes. The water surrounding the cell was changed every twelve hours.

The clay particles migrated toward the side of the container adjacent to the positive electrode whereas some of the *iron* was removed from the clay complex and deposited as a brown ferric hydroxide on the wall of the container that was against the cathode.

Titration of the clay acid with the hydrogen electrode

A part of the dialysed clay was so diluted that each milliliter contained 1 mgm. of solid. To 50 ml. of the suspension varying amounts of 0.0981 *N* potassium chloride were added in 0.05-ml. portions from an accurately calibrated 10-ml. burette, provided with a very fine tip so that amounts accurate to within 0.01 ml. could be obtained. The solution was stirred rapidly with a motor-driven stirrer during the process of titration. It was observed that even though considerable iron had been removed by electrodialysis there was a sufficient quantity displaced by 2 ml. of the potassium chloride solution to cause poisoning of the hydrogen electrode; therefore, some other means of measuring the pH had to be sought.

[2] Svedberg and Nichols (13) observed the size of clay particles of the same origin and history to be about 40$\mu\mu$.

The authors acknowledge their indebtedness to Richard Bradfield of the University of Missouri for supplying the clay suspension used in these experiments.

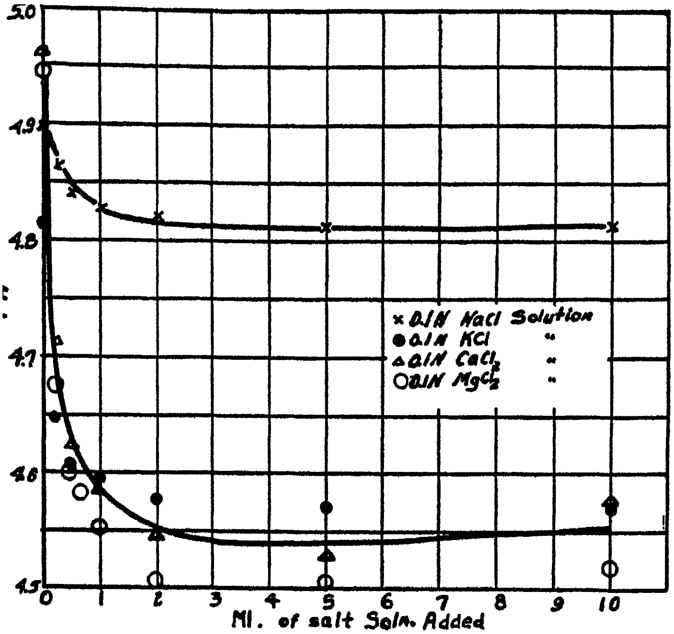

FIG. 1. THE EFFECT OF VARIOUS SALT SOLUTIONS ON THE pH OF A CLAY SUSPENSION

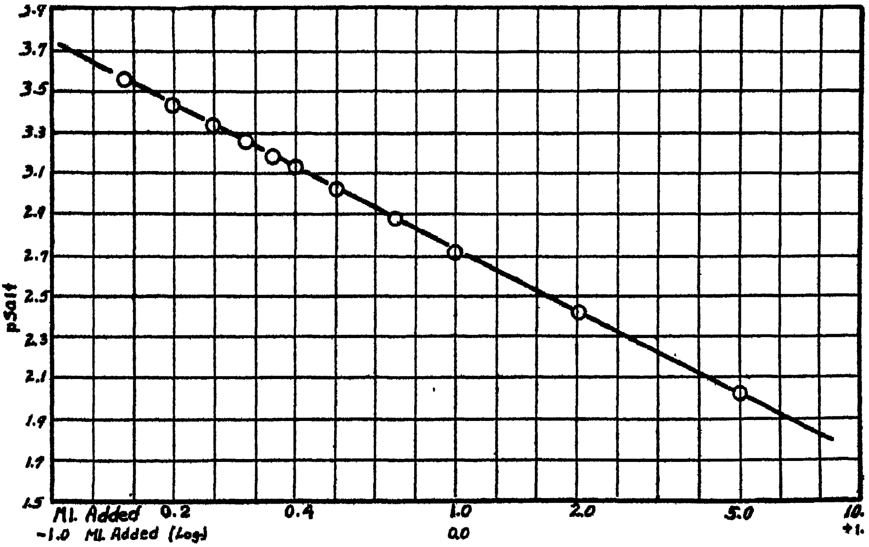

FIG. 2. THE ACCURACY OF THE METHOD IS INDICATED BY THE STRAIGHT LINE RELATION BETWEEN THE pSalt (ANALAGOUS TO pH) AND THE LOGARITHM OF THE AMOUNT OF SALT ADDED

Preparation and use of the quinhydrone electrode

Biilmann (1) has used the quinhydrone electrode in the study of soils with very good results. It was tried for this work and was found to be very satisfactory, as all the solutions were on the acid side of the neutral point. The quinhydrone electrode was calibrated in an acid phthalate solution against a hydrogen electrode, the circuit containing the salt bridge used in the actual measurements. The experiments were carried out at a temperature of 18°. The direct determination of the base-ion concentrations would be very desirable, but the experimental difficulties combined with the short time available have made these measurements impracticable at present.

TABLE 1

Titration of acid clay with 0.1 normal NaCl solution

SALT SOLUTION ADDED	EQUATION D				EQUATION E			
	Run No. 1		Run No. 2		Run No. 1		Run No. 2	
	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*
ml.								
0.05								
0.10	1.28	1.05	1.37	1.105	1.34	0.328	1.38	0.621
0.15								
0.20	1.44	0.682	1.39	0.278	1.41	0.0437	1.38	0.259
0.25								
0.30			1.60	0.183			1.09	$a > x$
0.35								
0.40	1.68	0.124	1.29	0.210	1.53	0.0593	1.40	$a > x$
0.50								
0.70	1.51	0.252	1.39		1.51	0.00582	1.42	0.0287
1.00								
2.00	1.58	0.220			1.35	$a > x$	1.34	$a > x$
5.00								
10.00	1.53	0.154	1.34	0.312	1.53	0.00672	1.36	$a > x$
Mean	1.50	0.413	1.39	0.386	1.44		1.41	
$H_H = 6.8 \times 10^{-6}$, $K_{Na} = 1.7 \times 10^{-5}$					This equation does not hold for NaCl			

The large dissociation constant of the Na salt of clay raises the charge on the clay micelles increasing the dispersion and incidentally holding back the H ion from being completely replaced.

Titration of acid clay with salt solutions

The data for the electrometric titration of the acid clay with various salt solutions are given in figures 1 and 2 after the pH is corrected for the diluting effect of the added solvent. Equilibrium was quickly reached after each addition. For a titration the initial volume was 50 ml. containing 50 mgm. of solid acid clay in suspension.

The results for the *a* and K*ex* values calculated from the data obtained, in pairs, using equation (D) and (E) are given in the tables, in addition to those calculated on the assumption that each clay group acts as a dibasic acid making exponent *p* in equations (F) and (G) equal to 2.

TABLE 2

Titration of acid clay with 0.1 normal KCl solution

SALT SOLUTION ADDED	EQUATION D				EQUATION E			
	Run No. 1		Run No. 2		Run No. 1		Run No. 2	
	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*
ml.								
0.05	2.53	0.593	1.54	0.129	2.21	0.095	2.50	0.070
0.10								
0.15								
0.20	1.28	0.166	2.05	0.229	3.85	0.037	1.83	0.0132
0.25								
0.30	4.17	0.117	1.84	0.237	3.33	0.0105	1.83	0.0110
0.35								
0.40	4.60	0.091	3.23	0.331	3.66	$a > x$	2.28	0.0063
0.50								
0.70	4.90	0.463	2.13	0.198	3.23	$a > x$	1.94	0.0076
1.00	3.25	0.379	2.07	0.450	1.17	$a > x$	2.04	0.0081
2.00								
5.00								
10.00	2.29	0.287	1.84	0.418	3.02	$a > x$	1.93	0.00032
Mean	3.43	0.299	2.10	0.332	2.92		2.05	
$H_H = 6.8 \times 10^{-6}$, $K_K = 1.15 \times 10^{-6}$					This equation does not hold for KCl			

The value *a* is the number of mols of replaceable H ion per liter of suspension, no matter whether one or more H's are attached to each clay molecule. Calculation by either of the alternate formulas gives the same value within a deviation corresponding to a rather small experimental error for the exchanges between the potassium, magnesium, or calcium ions and the H ion. The average is 2.87×10^{-5} as compared with 1.44×10^{-5} for the sodium ion. As the sodium compound has a much higher ionization constant, the charge on the clay particles is greater than with the other three ions, resulting in more dispersion and also in more attraction to prevent the loss of more hydrogens. This is probably the reason for the low *a* values in this determination.

TABLE 3

Titration of acid clay with 0.1 normal $MgCl_2$ solution

SALT SOLUTION ADDED	EQUATION E				EQUATION G			
	Run No. 1		Run No. 2		Run No. 1		Run No. 2	
	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*	$a \times 10^5$	$Kex \times 10^6$	$a \times 10^5$	$Kex \times 10^6$
ml.								
0.05								
0.10	2.15	0.588	1.71	0.893	1.925	2.31	1.31	1.68
0.15								
0.20	2.38	0.534	2.77	0.297	4.40	1.77	2.59	1.25
0.25								
0.30	2.74	0.331	3.00	0.301	4.93	1.99	4.30	1.29
0.35								
0.40	3.24	0.168	3.57	0.133	1.18	2.30	5.14	1.49
0.50								
0.70	2.92	0.622	3.71	0.138	3.53	2.74	6.43	1.62
1.00								
2.00	3.08	0.546	3.52	0.393	3.93	4.72	1.29	1.40
5.00								
10.00	2.96	0.722	3.53	0.448	2.61	$a > x$	2.41	3.79
Mean	2.783	0.501	3.267	0.401	3.25	2.81	3.35	2.09
	$K_H = 6.8 \times 10^6$, $K_{Mg} = 7.3 \times 10^6$				If $K_H = 6.8 \times 10^6$ and if $(K_{H_1})^8/K_{Mg_{1,2}} = Kex_G$, then $K_{Ca_{1,2}} = 1.9 \times 10^{-38}$			

The *Kex* values can be used with that of K_{H_1}—the dissociation constant of the monobasic clay acid, for which that suspension of clay having the highest pH was used—together with the mean *a* value, to calculate the dissociation constants for the four clay salts as listed at the bottoms of the tables. If the clay is dibasic, by assuming that the $K_{H_{1,2}}$ is equal to $(K_{H_1})^4$, an evaluation of the constants $K_{Mg_{1,2}}$ and $K_{Ca_{1,2}}$ can be made. They are of the order of 10^{-38}. Calcium and magnesium are not attached appreciably more tightly to the clay particles than potassium is, as evidenced by numerous published analyses of soils and of drainage waters. Hence the conclusion seems justified that these two ions do *not* replace two H ions from a single clay molecule but from two molecules and that this clay, under the conditions of the experiment, behaves as a monobasic acid. The failure of equation (F) to hold for sodium and potassium points strongly toward the same conclusion. The smaller *a* value for the sodium determinations may be explained if the greater degree of ionization of this salt is remembered. A larger charge is placed on the clay suspension thereby, and the remaining hydrogens which are readily replaced by the other three basic ions are held back.

TABLE 4

Titration of acid clay with 0.1 normal $CaCl_2$ solution

SALT SOLUTION ADDED	EQUATION E				EQUATION G			
	Run No. 1		Run No. 2		Run No. 1		Run No. 2	
	$a \times 10^5$	*Kex*	$a \times 10^5$	*Kex*	$a \times 10^5$	$Kex \times 10^6$	$a \times 10^5$	$Kex \times 10^6$
ml.								
0.05								
0.10	1.73	0.724	2.17	0.882	1.06	1.20	2.35	1.63
0.15								
0.20	2.17	0.120	2.83	0.267	1.10	1.46	5.23	1.13
0.25								
0.30			3.01	0.248			3.84	1.05
0.35								
0.40	2.46	0.271	2.65	0.298	1.95	2.85	7.00	1.25
0.50								
0.70	2.55	0.319	3.07	1.39	3.78	4.77	2.81	1.55
1.00								
2.00	2.69	0.540	3.07	0.581	2.81	$x > a$	3.60	1.20
5.00								
10.00	2.72	0.405	3.19	0.400	2.01	$x > a$	2.61	3.34
Mean	2.389	0.413	2.857	0.581	2.21	2.57	3.92	1.59

If $K_H = 6.8 \times 10^6$, $K_{Ca} = 7.6 \times 10^6$

If $K_H = 6.8 \times 10^6$
and if $(K_{H_1})^8/K_{Ca_{1,2}} = Kex_G$,
then $K_{Ca_{1,2}} = 2.2 \times 10^{-38}$

The clay suspension used contained a known weight of solid material (1 gm. of clay dried to constant weight at 100°). The equivalent weight of the clay acid as calculated, using the *a* values from the last six titrations, appears to be close to 3500 for clay dried in the oven, and about 3900 when dried in a vacuum desiccator. Since the evidence points strongly to the clay acid being monobasic, it seems very probable that this is also the molecular weight.

SUMMARY

1. Formulas are developed for base exchange in soils and in similar systems in which the equilibrium constant is shown to be a function of the ionization constants of the combination between the base ions and the clay.

2. Data obtained by titrating an acid clay suspension with solutions of salts in the presence of a quinhydrone electrode are substituted in the several equations and are apparently of sufficient accuracy to warrant certain conclusions in spite of the multiplication of the experimental error during the computations.

3. The base ions replace only one hydrogen from each clay "molecule," according to the evidence adduced. In other words, the clay behaves like a monobasic acid in contact with the solutions of neutral salts used.

4. The molecular weight of the clay is probably about 3500 when oven-dried and about 3900 when desiccator-dried at ordinary temperatures.

5. The ionization constants for the sodium, potassium, magnesium, and calcium salts of the clay used are calculated to be, when multiplied by 10^5, 1.70, 1.15, 0.73 and 0.76, respectively.

6. The exchange constants between sodium, potassium, magnesium, and calcium, and hydrogen are evaluated.

7. The method offers considerable promise of usefulness in laying down a rather exact physico-chemical ground work for base exchange and similar soil problems.

REFERENCES

(1) Biilmann, E. 1924 The measurement of the hydrogen ion concentration in soils by the quinhydrone electrode. *Jour. Agr. Sci.* 14: 232.

(2) Bjerrium, N. 1923 Dissociation constants of polybasic acids and their application to the calculation of molecular dimensions. *Ztschr. Phys. Chem.* 106: 219–242.

(3) Bradfield, R. 1923 The nature of the acidity of the colloidal clays of acid soils. *Jour. Amer. Chem. Soc.* 45: 2669–2678.

(4) Ehrenberg, P. 1909. The exchange of base in the salts of the so-called "weak acids" of the soil. *Landw. Jahrb.* 38: 857; *Chem. Abs.* 4: 1521.

(5) Jones, C. P. 1924 Adsorption and absorption of bases by the soil. *Soil Sci.* 17: 255–273.

(6) Kappen, H. 1916 Causes of acidity in soils which are acid through exchange of ions. *Landw. Vers. Sta.* 89: 39–80; *Jour. Chem. Soc.* 110 (I): 876.

(7) Kornfeld, G. 1917 Base exchange in permutite. *Ztschr. Elektrochem.* 23: 173–177.

(8) Kornfeld, G., and Rothmund, V. 1917 Base exchange in permutite. *Phys. Ztschr.* 18: 113–114.

(9) Liesengang, H. 1922 The exchange acidity of mineral soils. *Landw. Vers. Sta.* 99: 191.

(10) Page, H. J. 1926 The investigations of K. K. Gedroiz on base exchange and absorption. *Trans. Second Comn. Internatl. Soc. Soil Sci.* A: 208–231.

(11) Rothmund, V., and Kornfeld, G. 1918 Base exchange in permutite, I. *Ztschr. Anorgan. u. Allg. Chem.* 103: 129.

(12) Rothmund, V., and Kornfeld, G. 1919 Base exchange in permutite, II. *Ztschr. Anorgan. u. Allg. Chem.* 108: 215.

(13) Svedberg, T., and Nichols, C. 1923 Determination of the size and distribution of size of particles by centrifugal methods. *Jour. Amer. Chem. Soc.* 45: 2910–2917.

CROSS INOCULATION WITH COWPEA AND SOYBEAN NODULE BACTERIA[1]

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Many instances of abundant nodule formation have been observed on soybean plants growing in Illinois where it has been impossible to trace the source of the nodule bacteria to artificial inoculation or to the blowing and washing of inoculated soil upon the land. The explanation of these observations was not discovered until early in 1922, when C. D. Jones, working in this laboratory, found that a culture of soybean nodule bacteria produced nodules upon soybean and cowpea plants. Later in the same year Leonard (5) published a paper giving the results of investigations begun by him in 1917, in which he found "an interchangeability of the nodule-forming function between cultures of *B. radicicola* from soybean and cowpea nodules."

The laboratory observations of many investigators indicate that the nodule bacteria of the soybean plant are specific for this legume. Garman and Didlake (2), as well as Burrill and Hansen (1) concluded that the soybean nodule bacteria do not produce nodules on any other legume and belong, therefore, in a separate group. Burrill and Hansen (1) found, however, that the cowpea and soybean nodule bacteria have some similar growth habits on laboratory media. Löhnis and Hansen (8) and Shunk (9) likewise found that the cowpea and soybean nodule bacteria were similar in that they possessed monotrichous flagella, whereas bacteria belonging to groups other than the cowpea and soybean possess peritrichous flagella. Löhnis and Hansen (8) also found that both cowpea and soybean nodule bacteria cause little change in sterile milk, whereas members of all the other groups produce a serum zone at the top.

In field observations, Lewis and Nicholson (6) found no nodules on the roots of soybeans growing in the vicinity of well inoculated cowpeas. Lipman (7) and Hopkins (3) reported similar results from investigations conducted in New Jersey and Illinois, respectively. The absence of nodules on one variety of soybeans could not, however, be considered conclusive evidence that cowpea bacteria will not infect the soybean plant, for Voorhees (12) found that with two varieties of soybeans growing in the same field, one showed abundant nodule development while the other had none.

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[1] Contribution from the division of soil biology, department of Agronomy. Published with the approval of the director.

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According to Kirchner (4), Cohn observed nodule development upon the soybean grown in soil which had not received any kind of inoculation.

Recently Stevens (10, 11) and Wright (13) have shown that it is possible to divide the groups of nodule bacteria into strains or biotypes on the basis of serological reactions and cultural and physiological characteristics.

EXPERIMENTAL METHODS

In planning the experiments reported in this paper, it was recognized that the validity of the results secured by an investigation of this kind is to a large extent dependent upon the use of pure cultures. For this reason, extreme care was used to prevent any probability of error due to mixed cultures. Many of the cultures were secured from isolations made in this laboratory, but a few were obtained from other sources. In every case the bacteria were allowed to grow 6 to 10 days in modified Ashby's Solution. Portions of these liquid cultures were then shaken vigorously for 40 minutes with sharp sterilized sand to break up any clumps that might be present. These shaken cultures were diluted and plated in yeast-water mannitol agar (pH 6.8) and incubated 6 to 8 days at 28°C. Ten to thirty well isolated colonies were picked from each set of plates and tested for nodule production on cowpea and soybean plants. In several instances this procedure was repeated with individual cultures arising from these colonies. In order to obtain a further check upon the purity of the cultures, single cells were picked from 3 of the cultures by the Barber technique.

Pint jars were used for growing the test plants. The sand was washed and dried and about 2 gm. of 100-mesh limestone added to each jar before sterilizing. All of the seeds used were sterilized 4 to 5 minutes in a solution of bichloride of mercury (1:500) under a partial vacuum and then washed thoroughly with sterile water. Both cowpeas and soybeans were planted in the same jar. After the seed began to germinate, but before the plants were well up, 5 cc. of inoculum and 10 cc. of nutrient solution were added to each test jar. Dry sand was then spread over the surface to prevent the bacteria being carried from one jar to another by insects. The greenhouse was kept clean by frequent washings with a hose. The absence of nodules in the controls, used at the rate of 1 control to 3 inoculated jars, indicates that the great care taken to avoid contamination was effective. In this connection, it may be mentioned that in all cases of positive inoculation, the number of nodules per plant ranged from 12 to 35, further indicating that chance inoculation was not responsible for the nodules formed. The temperature was kept at 25 to 28°C. by placing the lower two-thirds of the jars in sand to which cool water could be added when necessary. The plants were allowed to grow about 21 days before they were examined for nodule development.

RESULTS

The data obtained in these investigations suggest an explanation for the differences in the opinions of other investigators on this subject. Table 1 shows that certain cultures isolated from the nodules of cowpeas produced nodules on the cowpea plant but not on the soybeans grown in the same jar, whereas certain others produced nodules on members of both plant groups under identical conditions. After this fact was discovered, repeated efforts were made to isolate from these cowpea cultures, colonies which would not

TABLE 1

Cross inoculation with soybean and cowpea nodule bacteria

	NUMBER OF COLONIES	TOTAL NUMBER OF TESTS	NODULATION	
			Cowpeas	Soybeans
Soybean 10[*]	6	42	+	+
Soybean 915	10	70	+	+
Soybean 20	3	21	+	+
Soybean 920a	10	70	+	+
Soybean 920b	3	21	+	+
Soybean 5[*]	5	14	+	+
Soybean 6[*]	3	21	+	+
Soybean 2[*]	1	4	+	+
Soybean 4	2	11	+	+
Soybean 8[*]	2	8	+	+
Soybean 15[*]	3	21	+	+
Soybean 840	3	12	+	+
Soybean 120	20	60	+	+
Soybean 121	14	28	+	+
Soybean 19	20	40	+	+
Cowpea 106[†]	3	11	+	
Cowpea 107[†]	3	11	+	
Cowpea 108[†]	3	11	+	
Cowpea 109[†]	3	11	+	
Cowpea 110[†]	3	11	+	
Cowpea 111	6	12	+	
Cowpea 112	6	12	+	
Cowpea 113	6	12	+	
Cowpea 114	4	8	+	
Cowpea 115	30	60	+	+
Cowpea 116	3	12	+	+
Cowpea 117	6	18	+	+
Cowpea 118	4	16	+	+

[*] Cultures obtained from W. H. Wright, University of Wisconsin. S. B. 8 and 10 were isolated from plants grown in Louisiana soil. S. B. 15 was isolated from plants grown on Florida soil. S. B. 2 came originally from U. S. Department of Agriculture. S. B. 5 and 6 came originally from Pennsylvania.

[†] Cultures obtained from A. L. Whiting, University of Wisconsin.

produce nodules on the roots of soybean plants. If cross-inoculation could be explained on the "mixed culture" hypothesis, it would seem that certain colonies could be found which would produce nodules on members of one plant group but not on the other. The number of colonies picked and tested was increased from 10 to 20 with one culture and to 30 with another culture. Each colony, however, gave good inoculation on both cowpea and soybean plants.

TABLE 2

Influence of dilution upon nodulation

DILUTION	JAR NUMBER	NUMBER OF NODULES PER PLANT			
		Culture 917		Culture 915	
		Cowpea	Soybean	Cowpea	Soybean
Undiluted	1	14	18	19	17
	2	16	12	13	No plants
1:100	1	12	8	14	10
	2	9	13	6	15
1:1,000	1	Diseased	9	5	5
	2	8	4	1	2
1:10,000	1	6	8	1	0
	2	4	3	Diseased	1
1:50,000	1	1	0	1	0
	2	0	1	0	0
1:1,000,000	1	1	0	0	0
	2	0	Diseased	0	0
1:10,000,000	1	0	0	0	0
	2	0	0	0	0
6 controls	1–6	0	0	0	0

About 20 cultures isolated from soybean nodules were tested, but extensive work was done with only 15 cultures. Each of the 103 colonies picked gave good inoculation on both of the host plants. All the single cells picked from soybean cultures 915, 917, and 920 produced nodules on the cowpea plant, as well as on the soybean.

After the difference in the nodule-producing power of the different cowpea cultures was noted, it seemed that the same difference might be expected in the behavior of various soybean cultures, but thus far no culture from the soybean nodule has been found to give negative results on either of the host plants under the conditions of these experiments.

It seemed probable, also, that if the cultures were mixed, the proportion of the respective organisms present would not be the same and that high dilutions might eliminate the members of one group and leave a sufficient number of those belonging to the other group to form nodules. Data secured from inoculations made in duplicate with 1 cc. of several dilutions from 2 cultures are given in table 2.

In the lower dilutions, an abundance of nodules was produced on all the healthy plants. In three pots a diseased condition of the plants existed, which was evidenced by the brown, club-end roots. These abnormal plants had no nodules in any case. At the higher dilutions, nodule development was rather irregular. In some cases in one jar of the pair the cowpea plants were sparsely inoculated whereas the soybean plants were without nodules, and in the duplicate jar the reverse condition was true. None of the plants in the 6 controls developed nodules. Since irregularities in nodule formation occurred with both species of the host plants at the same dilution of inoculum, it seems probable that either a pure culture of an organism interchangeable for inoculation purposes was used or that approximately the same number of cowpea and soybean nodule bacteria were present. Since, as previously stated, 30 well-isolated colonies produced cultures which inoculated both cowpea and soybean plants in every instance, the latter possibility seems the less likely of the two.

TABLE 3

Serological reaction of cowpea and soybean nodule bacteria

CULTURE	ANTI-SERUMS AND TITER			
	Cowpea 106	Cowpea 107	Soybean 120	Soybean 915
Cowpea 106	1:20,000	1:20,000	0	0
Cowpea 107	1:20,000	1:20,000	0	0
Cowpea 108	1:20,000	1:20,000	0	0
Cowpea 109	1:20,000	1:20,000	0	0
Cowpea 110	1:20,000	1:20,000	0	0
Cowpea 111	1:20,000	1:20,000	0	0
Cowpea 112	1:20,000	1:20,000	0	0
Cowpea 113	1:20,000	1:20,000	0	0
Cowpea 114	1:20,000	1:20,000	0	0
Soybean 2	0	0	1:20,000	1:20,000
Soybean 15	0	0	1:20,000	1:20,000
Soybean 120	0	0	1:20,000	1:20,000
Soybean 915	0	0	1:20,000	1:20,000
Soybean 920	0	0	1:20,000	1:20,000
Cowpea 115	0	0	0	0
Cowpea 116	0	0	0	0
Cowpea 117	0	0	0	0
Cowpea 118	0	0	0	0

Finally, cultures were re-isolated from nodules produced on the roots of cowpea plants by soybean cultures and tested again against both hosts. Nodules were produced in all cases. In isolations of this kind, the nodules were immersed 3 minutes in a solution of bichloride of mercury (1:500) and transferred to 70 per cent alcohol for 3 minutes. The alcohol was allowed to burn off. The nodules were then cut into halves with a sterilized scalpel, and organisms transferred from the center of the nodule to agar slants. When sufficient growth had occurred, the cultures were shaken with sand and plated as before.

In order to obtain further indications with reference to the possibility of mixed cultures, a number of agglutination tests were run. The technique used was practically the same as that described by Wright (13).

As is shown by table 3, the cowpea cultures which do not inoculate soybean plants are serologically the same. The ones which inoculate both hosts are in another group and are not agglutinated by antisera produced by the first group of cowpea cultures or by any of the soybean cultures tested.

If it is assumed that several strains of serologically unlike cowpea organisms exist, it is possible that an agglutination test might not be conclusive proof of the purity of the cultures. If in addition to the agglutination tests, however, the number of positive inoculation tests obtained with cultures secured from plate selection and single cell isolation is considered, the probability that mixed cultures explain this cross-inoculation phenomenon seems very remote.

DISCUSSION

It is believed that there are two fairly good reasons why one might suspect the possibility of cross-inoculation between nodule bacteria of the cowpea and soybean groups.

The first is that the two groups have many characters in common and differ alike from the nodule bacteria of all the other groups. The second reason is that certain strains of each group differ from other strains of the same group in cultural and physiological characteristics. In fact, one of these groups differs from the other group little, if any, more than a given strain within a group differs from certain other strains in the same group. It is not unreasonable, therefore, to expect that certain cultures may adapt themselves to either of the two hosts, whereas others are capable of infecting only one.

Furthermore, it is well-known that the cowpea nodule bacteria are more widely distributed in soils than are those of the other groups. The cowpea group contains the largest number of plant species of any common group. These species are more varied botanically than are the species of other groups. These facts suggest the possibility that the cowpea plant is capable of working in symbiosis with bacteria from the nodules of a great number of legumes which possibly do not in all cases cross-inoculate among themselves. This may explain why all of the soybean cultures tested possessed the power to produce nodules on the roots of cowpea, whereas some of the cowpea cultures infected the soybean plant and others did not. It will not be surprising, however, if soybean nodule bacteria are found which do not produce nodules upon the cowpea plant.

SUMMARY

1. Cultures of legume nodule bacteria obtained by plate selection and single cell isolation have produced nodules upon both cowpea and soybean plants.
2. Thirty-eight colonies picked from 9 different cultures of cowpea nodule bacteria produced an abundance of nodules upon the roots of 7 different varieties of the cowpea plant, but failed to produce nodules on the roots of any one of 7 varieties of soybean plants grown in the jars with the cowpeas.
3. Forty-three colonies picked from 4 different cultures of cowpea nodule bacteria produced an abundance of nodules upon 7 varieties each of cowpeas and soybeans.
4. One hundred and three colonies picked from 15 different cultures of soybean nodules bacteria produced an abundance of nodules upon the roots of 7 varieties each of cowpeas and soybeans.
5. Both soybean and cowpea plants produced many nodules when inoculated with 3 different cultures of soybean nodule bacteria obtained by single cell isolations.
6. No cultures obtained from soybean nodules were tested which failed to produce nodules upon the cowpea.

REFERENCES

(1) Burrill, T. J., and Hansen, R. 1917 Is symbiosis possible between legume bacteria and non-legume plants? Ill. Agr. Exp. Sta. Bul. 202.

(2) Garman, H., and Didlake, M. 1914 Six different species of nodule bacteria. Ky. Agr. Exp. Sta. Bul. 184.

(3) Hopkins, C. G. 1912 Nitrogen bacteria and legumes. Ill. Agr. Exp. Sta. Bul. 94.

(4) Kirchner, O. 1895 Die wurtzelknöllchen der Sojabohne. *Beitr. Biol. Pflanz.* 7: 213–223.

(5) Leonard, L. T. 1923 Nodule production kinship between the soybean and the cowpea. *Soil Sci.* 15: 277–283.

(6) Lewis, L. L., and Nicholson, J. F. 1905 Soil inoculation. Okla. Agr. Exp. Sta. Bul. 68.

(7) Lipman, J. G. 1911 Bacteria in relation to country life, p. 231. New York.

(8) Löhnis, F., and Hansen, R. 1921 Nodule bacteria of leguminous plants. *Jour. Agr. Res.*, 20: 543–554.

(9) Shunk, I. V. 1921 Notes on the flagellation of nodule bacteria of the leguminosae. *Jour. Bact.* 6: 239–242.

(10) Stevens, J. W. 1923 Can all strains of a specific organism be recognized by agglutination? *Jour. Infect. Diseases* 33: 557–566.

(11) Stevens, J. W. 1925 A study of various strains of *Bacillus radicicola* from nodules of alfalfa and sweet clover. *Soil Sci.* 20: 45–65.

(12) Voorhees, J. H. 1915 Variations in soybean inoculation. *Jour. Amer. Soc. Agron.* 7: 139–140.

(13) Wright, W. H. 1925 The nodule bacteria of soybeans: I. Bacteriology of strains. II. Nitrogen fixation experiments. *Soil Sci.* 20: 95–141.

THE RELATION OF ORGANIC MATTER AND NITROGEN CONTENT TO SERIES AND TYPE IN VIRGIN GRASSLAND SOILS[1]

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It is the general theory of the system of soil survey in use throughout the United States that the series and types into which soils are classified and mapped are fundamentally distinct units not only from the standpoint of their own properties but also from the standpoint of their relations to plant production. Field identification is of necessity almost entirely a physical matter, such factors as climatic environment, topographic position, physical characteristics of the profile, and to some extent rock origin and process of formation being the usual bases for series differentiation. Series are further divided into types principally on the single physical feature of the textural classification of the surface soil. The only direct aids toward chemical classification which as a rule are employed, are field tests for reaction and salinity. As far as the surveyor is concerned in the field, fundamental chemical differences are purely a matter of opinion based on gross features, such as organic matter which the eye can perceive, and on his knowledge concerning the effect of climate and rock origin on soil composition.

Soil scientists should be very much interested in all quantitative studies which reveal the degree of chemical or physical homogeneity of the various types, or which indicate the chemical peculiarities of the various series. Such studies, on the one hand, serve to inform the surveyor as to the accuracy of his correlation, and on the other and far more important hand, probe the soundness of the system and provide information for its improvement.

Organic matter has always been considered of high importance as a factor in series differentiation, originally on account of its well-known contribution to soil value. There seems to be another and more important reason, however, why it should be given consideration. Organic matter is produced from the plant material grown on the soil. The very climatic factors and soil differences which are used in distinguishing series and types determine in considerable part, the quantity of vegetation which the soil will produce. Likewise these same factors determine to an extent, the nature, rate, and completeness of organic matter decay, and the amount of its loss by erosion and leaching. Therefore, the amount of organic matter found in soil, particularly in its virgin state, should be, when properly interpreted, an important clue to the proper classification of the soil.

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[1] Contribution from the Department of Agronomy, Nebraska Agricultural Experiment Station. Published with the approval of the Director as Paper No. 42 Journal Series.

The major portion of the data in this paper has been taken from a thesis presented by the junior author in 1924 to the Graduate Faculty of the University of Nebraska in partial fulfillment of the requirements for the degree of Master of Science. Acknowledgment is made to H. E. Engstrom, E. B. Engle, H. E. Weakly, Glen Hunt, and G. M. Bahrt for assistance in the collection of soil samples, and to E. B. Engle and E. H. Tyner for analytical services. Acknowledgment is also made to Prof. W. W. Burr, Chairman of the Department of Agronomy and to Dr. G. E. Condra, Director of the State Soil Survey, for many valuable suggestions on the preparation of the manuscript.

In the use of this feature of soil in field classification, the surveyor is limited to three of its physical manifestations; namely, the blackness of the surface layer, the amount of fiber which the surface layer appears to contain, and the depth to which the surface layer extends. Although one may become very adept in the detection of variations in these respects and may feel confident that series distinctions may be based upon them as consistently as upon any other physical property, he has no assurance that the actual organic matter content is accurately estimated thereby. The chemist knows that these physical manifestations by themselves are at best very crude indices of actual organic matter content, and would rather suspect that the surveyor's success in mapping soil types as homogenous units is achieved in spite of the employment of these aids, and by reason of relations of the organic portion of soils to other properties of the series and type.

The purpose of this paper is to show the homogeneity of various soil types in organic matter and nitrogen content, also the differences among series in the same respects. The data presented indicate that certain factors are of outstanding importance in determining organic matter content, and the relation of these factors to soil classification is discussed.

HISTORICAL

Pendelton (11) has made a study of the uniformity of the soil type in California, as mapped by the Bureau of Soils. His object was to determine whether certain soil types judged to be the same in soil survey work in the field really are the same, or similar, when classified in the laboratory. He worked with 24 cultivated soils representing 4 soil types. He found total nitrogen varying markedly from type to type. The total nitrogen content of the several types was "reasonably constant within the type and rather distinct for the types." He does not discuss the relation of nitrogen to texture but found nitrogen varying directly with the amount of the "finer sediments present in the soil." His data show that types with the highest hygroscopic coefficients are highest in nitrogen content.

Walker (17) studied the chemical composition of 67 soils and 64 subsoils, representing 27 Louisiana soil types. None of his samples is described as being from virgin areas. His data do not indicate that the nitrogen content of either surface or subsoil is related to type, series, or texture. He states, "For all practical purposes—the soils are (*all*) uniform and very low—in nitrogen."

Blair and McLean (6) in a study of 24 soil types from 10 series in the Camden area of New Jersey, have observed that there is a gradual increase in total plant-food "following from the lighter to the heavier types through the different series." They have also observed that there is a "measure of similarity between samples of any particular type." Their data indicate that the above statements hold particularly for nitrogen and carbon.

De Turk (8) reports an exhaustive study of the variation in nitrogen, carbon, and phosphorus content in three Illinois soil types; namely, yellow gray silt loam, brown silt loam, and black clay loam represented by numerous 0 to 6⅔-inch and 6⅔ to 20-inch samples from widely scattered sections of the state. The samples had been collected and analyzed during the routine course of the Illinois soil survey. De Turk does not explain whether they were from virgin or cultivated fields. He states that as far as "individual samples are concerned, the variation within a given type, even in a given county is very wide,—the probable errors of individual samples ranging from 5.8 per cent to 19.5 per cent of the mean in a number of cases which were computed." He states also that "the extreme range of values as indicated by individual analyses would completely obliterate all chemical boundary lines between soil types." On the average, however, the carbon and nitrogen contents of the types when compared by sectional divisions of the state are quite distinct. De Turk divided the state into rough cardinal sections preserving the grouping by main soil areas, and shows that carbon and nitrogen in the 0 to 6⅔-inch section generally decrease in passing from north to south. This phenomenon he ascribes to the temperature and moisture relations in the south being more favorable to rapid decomposition of organic matter. A similar gradation was not found for phosphorus, nor for nitrogen and carbon in the 6⅔ to 20-inch section.

Brown and O'Neal (7), in a study on the correlation of color with organic matter, report the nitrogen and carbon content of 14 Iowa soil types each represented by composite samples from 4 to 16 counties. They do not discuss at any length the range in nitrogen and carbon in the different types but point out that the quantities of these constituents may overlap considerably in case of certain comparable loessial types, but may be quite different in extreme cases. Certain differences are ascribed to texture. They observe that there may be some relation between color and average organic matter content, but carbon and nitrogen contents may vary widely in individual samples without color changes being detectable. They do not state whether the samples studied were from virgin or cultivated areas.

Alway and associates did much work on the nitrogen content of the virgin prairie soils of Nebraska before soil surveys had been made of the areas studied. Alway and Trumbell (4) present data on the uniformity in nitrogen content of the 0 to 6-inch section of a number of virgin prairie fields in eastern, south central, and western Nebraska. The following summary of their data prepared by the writers shows the variation in nitrogen content between individual cores of soil. Alway and Trumbell believe that textural differences are mainly responsible for the wider variations in nitrogen content which are found between individual cores in the same field and between different fields in the same county.

COUNTY	NUMBER OF CORES	AVERAGE N CONTENT	RANGE	PROBABLE ERROR IN A SINGLE CORE
		per cent	*per cent*	*per cent*
Lancaster	35	0.290	0.250–0.317	±0.010
Thayer	33	0.248	0.213–0.268	±0.009
Thayer	32	0.248	0.205–0.292	±0.017
Chase	10	0.134	0.110–0.155	±0.009
Perkins	5	0.119	0.100–0.130	±0.008
Perkins	15	0.150	0.096–0.228	±0.028
Perkins	20	0.133	0.094–0.199	±0.019
Perkins	10	0.122	0.082–0.179	±0.024
Perkins	5	0.067	0.039–0.092	±0.014
Perkins	10	0.099	0.082–0.112	±0.006
Perkins	20	0.135	0.106–0.158	±0.012

Alway and McDole (3) studied virgin samples from six points in the prairie-covered loess soil region of Nebraska, beginning at Weeping Water where the annual precipitation is over 30 inches, and extending westward to Wauneta where the precipitation is about 18 inches. They interpreted their studies as indicating a transition in soil character as a result of climate. They found considerable decrease in the quantity of organic matter and nitrogen from Weeping Water westward. In the surface foot at Weeping Water the organic matter and nitrogen contents were 4.98 and 0.236 per cent, respectively, whereas at Wauneta the percentage of these constituents was 2.77 and 0.136, respectively. They state (3, p. 219) that "The differences in the nitrogen content of the first foot of the different areas might be due, partly or wholly, to differences in texture, a coarser soil tending to accumulate less organic matter. However, a comparison of the hygroscopic coefficients makes it evident that the texture does not account for the differences. The greater amount of nitrogen is probably due to the greater production of vegetable matter, which in turn is a consequence of the greater rainfall and lower evaporation."

Russel (13), working with Alway, has obtained, by dividing the hygroscopic coefficient by the nitrogen content, a measure of the relation of nitrogen to texture. Seventy 0 to 6-inch samples from Ramsey County, Minnesota, gave hygroscopic coefficient-nitrogen (*HC*/N) ratios ranging from 28 to 57 and averaging 38.4. Thirty-five of these gave values ranging between 37 and 43 and averaging 39.8. Only a few of these samples were virgin soils, the others being from cultivated fields in average condition of productivity. In discussing these findings, he states, "It must not be concluded that the nitrogen content of a soil can be calculated by dividing the hygroscopic coefficient by 38.4 or by any other number. . . . . However if there be a general relation between nitrogen and hygroscopic coefficient the determination of these values (*HC*/N ratios) may give valuable information about a soil. Thus, if nitrogen is found to be lower than indicated by formula, the soil is to be judged as relatively low in nitrogen, and if it is higher than the calculated, it is in a high state of fertility with respect to that element. In advising such a formula, it is of course to be recognized that since the nitrogen content decreases with the depth, a different formula would be required for each section sampled."

Later, Stump (16), under the direction of Russel, studying the *HC*/N ratios of 15 virgin fields in Reno County, Kansas, found the ratios of 0 to 7-inch samples to be practically constant and averaging 43. Subsurface (7 to 20 inches), and subsoil (20 to 40 inches), gave widely varying ratios.

Sievers and Holtz (15) in Washington have made valuable contributions to our knowledge concerning the relationships of organic matter and nitrogen to climate and other factors, and have presented data showing plainly the tendency of these soil constituents to be lower in quantity in the coarser textured soils, and in the regions of lower rainfall.

EXPERIMENTAL

Collecting the samples

In grassland soils, the nitrogen and organic matter content decreases regularly with depth (14) apparently independently of physical appearances of horizonation. Therefore samples were taken to empirical depths rather than by horizons, the thickness of which would vary widely. Furthermore, the bulk of the organic matter is confined to the surface foot. Accordingly only the surface foot of soil was sampled, always in two 6-inch sections. Data are reported on 334 such samples all taken from virgin areas—either meadows, clean roadsides, or pastures which had never been plowed or overgrazed. Except in a few cases, the samples are composites of ten cores taken ten paces apart in a straight line over a uniform portion of the area being sampled. In a limited number of cases as few as seven cores have been composited. Sampling was done with a pair of augers or a tube, care being taken to sample to exact depths and to avoid contamination of the lower sample by the surface soil above. Aerial vegetation and debris were always swept away before sampling, but all roots and undecayed matter removed with the sample were included in it.

All but 12 of these samples were accumulated with this study in mind during 6 years. Soil survey maps were carefully followed in selecting the areas, except in a few cases where surveys were unavailable; there, the types were identified through acquaintance with them elsewhere. Only typical areas within the type were sampled.

The data on 12 samples have been compiled from the work of Alway and McDole (3). These samples are composites of 20 cores by inches in 5 fields, in each of 6 areas. The areas sampled by them fit well into the plan of this study and their methods for determining carbon, nitrogen, and hygroscopic coefficient are strictly comparable with those used in this investigation. Since the publication of their work, detailed soil surveys have been made in each area, which makes the identification of the soil types possible.[2]

Soil types studied

Figure 1 shows the location of the 147 fields sampled in Nebraska. These fields represent 18 series and 35 types. Ten series represented by 15 types are from uplands; 5 series and 9 types are from terraces; and 3 series and 11 types are from bottom land. In addition, 10 areas identified as Pratt loam were sampled in central Kansas, and 5 areas each of Clarion and Hemsted silt loam were sampled in Minnesota.

Relation of organic matter to nitrogen

A number of investigators have shown that the ratio of organic matter to nitrogen in soil varies between narrow limits. Alway (1) and Fraps (9) have both suggested that for many purposes the organic content of a soil may be estimated by multiplying the nitrogen content by a factor. Read and Ridgell (12) have concluded that organic content may be calculated more closely from a nitrogen than from a carbon factor. Alway and Vail (5) found

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[2] The Alway-McDole samples which will hereafter be referred to as the *A* samples have been designated as follows:

A-WW	Weeping Water area	Carrington silt loam
A-L	Lincoln area	Carrington silt loam
A-Ha	Hastings area	Grundy silt loam
A-Ho	Holdrege area	Holdrege loam
A-Mc	McCook area	Holdrege silt loam
A-Wa	Wauneta area	Holdrege loam

the organic matter-nitrogen ratio (C × 1.724/N) in the first and second 6-inch sections of Nebraska virgin prairie soils to vary between 17.6 and 18.5. Alway and McDole (3) found organic matter-nitrogen ratios averaging 21.2 for the first 6-inch section, and 19.4 for the second 6-inch section, and varying but little from eastern to western Nebraska.

Tables 1 and 2 show the organic matter-nitrogen ratios of a limited number of samples selected as representative of the range of textures included in this study. Included in these tables are the samples of Alway and McDole. The data for the 0 to 6-inch sections are shown in table 1, and for the 7 to 12-inch sections in table 2. The soils are arranged in descending order of hygroscopicity.

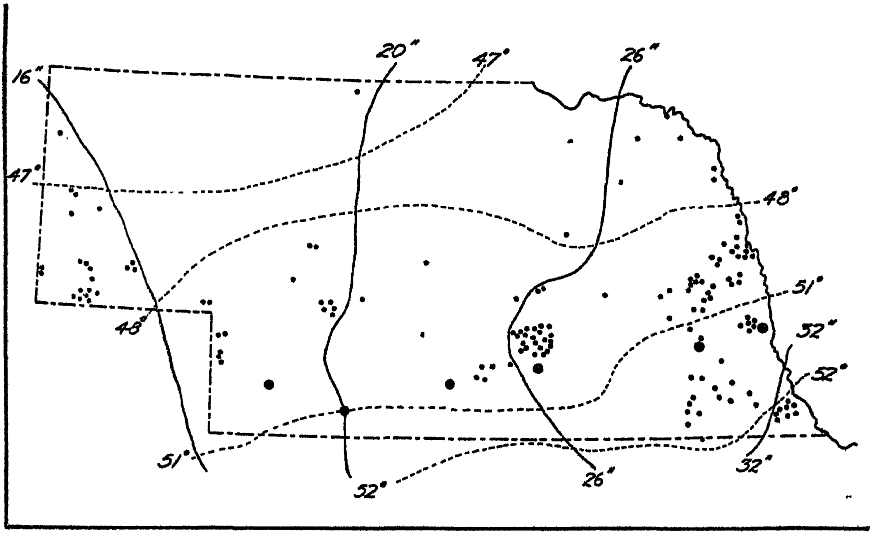

FIG. 1. MAP SHOWING THE LOCATION OF ALL AREAS SAMPLED IN NEBRASKA, WITH MEAN ANNUAL PRECIPITATION AND TEMPERATURE LINES

The six large circles indicate the location of the Alway-McDole areas

The average organic matter-nitrogen ratios for the 0 to 6 and 7 to 12-inch samples are 20.8 and 19.2, respectively. These averages are very close to the averages found by Alway and McDole and are but very little altered if the *A* samples be excluded. The average deviation from the mean in the 0 to 6-inch samples is ±1.3 and the probable error of a single ratio is ±1.9. For the 7 to 12-inch samples the deviation from the mean ratio is ±1.1. The variation in the organic matter-nitrogen ratios among the lighter textured samples in the lower half of both tables is no wider than in the tables as a whole.

For practical purposes the organic content in the surface foot of a virgin Nebraska grassland soil may be considered to be approximately twenty times the nitrogen content. In a study of the relation of organic matter and nitrogen to type, it is apparent that the same general conclusions would be reached by direct determination of either organic matter or nitrogen. Therefore on the samples not included in tables 1 and 2, nitrogen only has been determined and all conclusions to follow are based on that determination.

TABLE 1

Relation of organic matter (C × 1.724) to nitrogen for selected 0–6-inch samples

SAMPLE NUMBER	SOIL TYPE	COUNTY	HYGROSCOPIC COEFFICIENT	ORGANIC MATTER	NITROGEN	$\frac{\text{O.M.*}}{\text{N}}$
				per cent	*per cent*	
2743	Carrington silt loam	Cass	14.0	6.48	0.296	21.9
2750	Marshall silt loam	Saunders	13.3	5.67	0.300	18.9
2771	Carrington silt loam	Saunders	12.5	5.98	0.298	20.0
2694	Marshall silt loam	Cass	12.3	5.51	0.261	21.1
2736	Carrington silt loam	Cass	12.3	6.55	0.285	22.9
2764	Carrington silt loam	Saunders	12.0	4.74	0.271	17.5
2722	Marshall silt loam	Saunders	11.8	5.05	0.267	18.9
2715	Carrington silt loam	Cass	11.6	5.46	0.254	21.4
2729	Marshall silt loam	Butler	11.5	5.53	0.274	20.2
2785	Carrington silt loam	Richardson	11.5	5.20	0.254	20.5
2757	Carrington silt loam	Saunders	11.4	4.91	0.257	19.1
A-L	Carrington silt loam	Lancaster	11.3	5.84	0.264	22.1
2528	Carrington silt loam	Richardson	11.3	5.86	0.260	22.5
2708	Marshall silt loam	Cass	11.2	5.84	0.251	23.3
A-WW	Carrington silt loam	Cass	11.2	5.74	0.266	21.6
2690	Colby silt loam	Hall	10.8	2.89	0.147	19.7
2549	Marshall silt loam	Richardson	10.7	5.88	0.216	27.2
2778	Marshall silt loam	Richardson	10.7	4.58	0.259	17.7
2542	Marshall silt loam	Richardson	10.6	4.67	0.225	20.7
2701	Marshall silt loam	Cass	10.6	5.58	0.254	21.9
1316	Lamoure clay loam	Hall	10.0	4.19	0.203	20.6
2536	Carrington silt loam	Richardson	9.9	4.76	0.223	21.3
A-Ho	Holdrege silt loam	Phelps	9.9	5.05	0.231	21.8
2646	Hall silt loam	Hall	9.2	4.92	0.235	21.0
A-Ha	Grundy silt loam	Adams	9.2	4.77	0.219	21.8
2669	Hall v.f.s. loam	Hall	8.7	4.60	0.206	22.3
A-Mc	Holdrege silt loam	Red Willow	8.6	3.24	0.162	20.0
A-Wa	Holdrege loam	Chase	8.3	2.17	0.159	19.9
2623	O'Neill loam	Hall	7.4	3.44	0.188	18.2
2677	Cass sandy loam	Hall	6.4	3.55	0.183	19.4
2673	Hall sandy loam	Hall	4.7	2.75	0.131	21.0
2632	O'Neill sand	Hall	2.9	1.23	0.068	18.1

Average of *A* samples	21.2
Average of all samples, exclusive of *A* samples.	20.7
Average of all samples	20.8

* O.M. = organic matter.

Importance of hygroscopic coefficients

At the outset, it was felt that, in an investigation involving the comparison of nitrogen contents in many different soil types and from many different sections, the factor of texture would probably overshadow and conceal all

TABLE 2

Relation of organic matter (C × 1.724) to nitrogen for the 7–12-inch samples, corresponding to those in table 1

SAMPLE NUMBER	SOIL TYPE	COUNTY	HYGROSCOPIC COEFFICIENT	ORGANIC MATTER	NITROGEN	$\frac{O.M.^*}{N}$
				per cent	*per cent*	*per cent*
2758	Carrington silt loam	Saunders	15.3	3.96	0.205	19.3
2744	Carrington silt loam	Cass	14.2	4.76	0.240	19.9
2723	Marshall silt loam	Saunders	14.0	3.76	0.208	18.1
2751	Marshall silt loam	Saunders	14.0	4.27	0.220	19.4
2772	Carrington silt loam	Saunders	13.9	4.21	0.219	19.2
2737	Carrington silt loam	Cass	13.3	4.60	0.223	20.6
2765	Carrington silt loam	Saunders	13.3	3.98	0.204	19.5
A-L	Carrington silt loam	Lancaster	12.6	3.77	0.187	20.1
2709	Marshall silt loam	Cass	12.6	4.08	0.223	18.3
A-WW	Carrington silt loam	Cass	12.3	3.84	0.200	19.2
2702	Marshall silt loam	Cass	12.3	3.76	0.192	19.6
2695	Marshall silt loam	Cass	12.2	3.81	0.205	18.6
2730	Marshall silt loam	Butler	12.0	4.36	0.224	19.5
2716	Carrington silt loam	Cass	11.9	4.22	0.207	20.4
2529	Carrington silt loam	Richardson	11.3	4.08	0.189	21.5
2786	Carrington silt loam	Richardson	11.2	3.94	0.189	20.9
2536	Carrington silt loam	Richardson	11.0	4.24	0.205	20.7
2779	Marshall silt loam	Richardson	10.7	3.53	0.190	18.6
2691	Colby silt loam	Hall	10.7	1.15	0.063	18.3
2543	Marshall silt loam	Richardson	10.3	3.13	0.170	18.4
2550	Marshall silt loam	Richardson	10.3	2.98	0.159	18.8
H-Ho	Holdrege silt loam	Phelps	10.2	2.75	0.139	19.8
A-Mc	Holdrege silt loam	Red Willow	10.0	2.08	0.107	19.5
1317	Lamoure clay loam	Hall	9.8	1.72	0.099	17.4
A-Ha	Grundy silt loam	Adams	9.7	2.74	0.143	19.1
A-Ho	Holdrege loam	Chase	8.8	1.99	0.106	18.8
2647	Hall silt loam	Hall	8.5	2.67	0.133	20.1
2670	Hall v.f.s. loam	Hall	8.5	2.98	0.146	20.4
2624	O'Neill loam	Hall	6.4	1.81	0.090	20.1
2678	Cass sandy loam	Hall	5.0	1.66	0.098	17.0
2674	Hall sandy loam	Hall	3.4	1.10	0.059	18.7
2633	O'Neill sand	Hall	2.5	0.60	0.038	15.9

Average *A* samples..................	19.4
Average of all, exclusive of *A* samples.	19.2
Average of all samples...............	19.2

* O. M. = organic matter.

the other factors, unless by some method, it could be kept constant, or a correction could be made for it. As one of the authors (13) had previously developed the idea of expressing the texture-nitrogen relationship by means of the hygroscopic coefficient-nitrogen (HC/N) ratio, it was decided that the same method should be used in this work.

The hygroscopic coefficient will be used in this study as an expression of texture without entering into a discussion of its merit. A series of soils arranged in descending order of hygroscopic coefficient will be considered as ranging in texture from heavy to light, that is, from fine to coarse. No attempt will be made in this paper to discuss the relation of hygroscopic coefficient to soil type and class. For the hygroscopic coefficient determinations, the methods of Alway, Klein, and McDole (2) were employed.

HOMOGENEITY OF SOIL TYPES IN NITROGEN CONTENT

Table 3 shows the nitrogen contents and hygroscopic coefficients of the 0 to 6-inch and 7 to 12-inch samples of 14 Nebraska soil types[3] represented in this study by as many as four fields each. The table also shows HC/N ratios, and the calculated probable error of a single ratio for each type.

All types exhibit more or less heterogeneity in texture. They likewise exhibit a generally corresponding heterogeneity in nitrogen content. This apparent lack of uniformity disappears to some extent in all but the last three soils, when the ratios of hygroscopic coefficient to nitrogen are calculated. For example, in Carrington silt loam, the HC/N ratios of the 0 to 6-inch samples vary from 39.0 to 49.5 and average 44.2. The probable error of a single ratio is ±1.9. Knowing the hygroscopic coefficient, one might calculate the nitrogen content by dividing the hygroscopic coefficient by 44.2. At the widest the calculated value might depart from the actual analysis by 0.035 per cent, with an even chance that the departure would be within 0.011 per cent. In the 7 to 12-inch section, the HC/N ratios vary from 53.7 to 71.4, with an even chance that any ratio is found within the range 63.1 ±3.4.

The variability in HC/N values in both the first and second 6-inch sections is proportionally much the same in all types, excluding the last three. The probable error is ±4 to 6 per cent of the mean for the first 6 inches, and ±6 to 10 per cent of the mean for the second 6 inches. Rosebud silt loam or Bridgeport very fine sandy loam in the short-grass region of extreme western Nebraska is as homogenous in nitrogen content on an equivalent texture basis as is Carrington or Waukesha silt loam in the tall-grass section of eastern Nebraska, and Valentine fine sand in the Sand Hills Section of the state is as uniform as either. The three types of the Waukesha, Hall, and O'Neill series, which are all terrace formations, are as homogenous as the upland types.

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[3] The description and distribution of these soil types in Nebraska may be found in Nebraska Conservation and Soil Survey Bulletin 15, the soil resources of Nebraska, by G. E. Condra, 1920.

TABLE 3

Homogeneity in nitrogen content of Nebraska soil types represented by four or more samples

0-6-INCH SAMPLE NUMBER	COUNTY	0-6-INCH			7-12-INCH		
		Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
			per cent			*per cent*	
Carrington silt loam							
2743	Cass	14.0	0.296	47.3	14.2	0.240	59.1
3400	Lancaster	12.8	0.273	46.8	14.0	0.202	69.3
2771	Saunders	12.5	0.298	42.0	13.9	0.219	63.4
2736	Cass	12.3	0.285	43.1	13.3	0.223	59.6
2934a	Johnson	12.2	0.253	48.2	13.2	0.185	71.4
2491	Gage	12.0	0.278	43.2	13.1	0.216	60.7
2617	Seward	12.0	0.254	47.3	13.0	0.203	64.0
2764	Saunders	12.0	0.271	44.3	13.3	0.204	65.2
1139.1	Gage	11.7	0.275	42.5	12.5	0.203	61.5
2715	Cass	11.6	0.254	45.7	11.9	0.207	57.4
2785	Richardson	11.5	0.254	45.3	11.2	0.189	59.2
1381	Saunders	11.5	0.295	39.0	12.7	0.212	59.9
2757	Saunders	11.4	0.257	44.3	15.3	0.205	74.6
A-L	Lancaster	11.3	0.264	42.8	12.6	0.187	67.3
2528	Richardson	11.3	0.260	43.5	11.3	0.189	59.8
2856	Otoe	11.3	0.242	46.6	11.9	0.190	62.7
2574	Johnson	11.2	0.269	41.5	12.4	0.193	64.2
A-WW	Cass	11.2	0.266	42.1	12.3	0.200	61.5
1373	Burt	10.8	0.218	49.5	11.2	0.157	71.3
2535	Richardson	9.9	0.223	44.4	11.0	0.205	53.7
1141.1	Gage	9.9	0.251	39.4	10.5	0.173	60.7
1140.1	Gage	9.3	0.221	42.0	11.0	0.179	61.5
22 samples, average.......		11.5	0.262	44.2	12.5	0.199	63.1
Probable error of a single variant............				±1.9	......	....	±3.4
Marshall silt loam							
2750	Saunders	13.3	0.300	44.3	14.0	0.220	63.6
2694	Cass	12.3	0.261	47.1	12.2	0.205	59.5
2839	Cedar	12.0	0.263	45.6	11.1	0.131	84.8
2722	Saunders	11.8	0.267	44.2	14.0	0.208	67.3
1393	Saunders	11.6	0.244	47.5	12.9	0.173	74.6
2817	Thurston	11.5	0.231	49.8	11.1	0.143	77.7
2729	Butler	11.5	0.274	41.9	12.0	0.224	53.6
2708	Cass	11.2	0.251	44.6	12.6	0.223	56.5
2878	Washington	11.1	0.235	47.2	10.8	0.180	60.0
2549	Richardson	10.7	0.216	49.5	10.3	0.159	64.8
2778	Richardson	10.7	0.259	41.3	10.7	0.190	56.3

TABLE 3—*Continued*

0-6-INCH SAMPLE NUMBER	COUNTY	0-6-INCH			7-12-INCH		
		Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
			per cent			*per cent*	
Marshall silt loam—Continued							
2542	Richardson	10.6	0.225	47.1	10.3	0.170	60.6
2898	Washington	10.6	0.267	39.7	10.0	0.174	57.5
2701	Cass	10.6	0.254	41.7	12.3	0.192	64.0
2896	Douglas	10.3	0.255	40.4	11.2	0.194	57.8
2828	Dixon	10.1	0.229	44.1	10.0	0.142	70.5
16 samples, average........		11.2	0.252	44.8	11.6	0.183	64.3
Probable error of a single variant.............				±2.1	......	....	±5.9
Grundy silt loam							
2960	Butler	13.0	0.306	42.5	13.5	0.208	64.9
3720	Gage	11.4	0.282	40.4	11.5	0.188	61.2
1138.1	Gage	11.2	0.272	41.2	10.0	0.178	56.2
2610	Seward	11.0	0.277	39.7	11.8	0.205	57.6
3308	Hall	9.9	0.259	38.2	10.4	0.189	55.0
2578	Johnson	9.7	0.266	36.3	9.2	0.174	52.8
3808	Howard	9.6	0.200	48.0	10.5	0.149	70.5
2456	Polk	9.4	0.256	36.8	9.9	0.183	54.0
A-Ha	Adams	9.2	0.219	42.0	9.7	0.143	67.9
2510	Hall	8.1	0.186	43.5	8.1	0.105	77.1
10 samples, average........		10.3	0.252	40.9	10.5	0.172	61.7
Probable error of a single variant.............				±2.3	......	....	±5.5
Rosebud silt loam							
2950	Kimball	10.1	0.174	58.0	13.1	0.098	133.7
4001	Cheyenne	10.0	0.170	58.8	10.3	0.109	94.5
2946	Kimball	10.0	0.164	61.0	10.2	0.096	106.3
3993	Cheyenne	9.4	0.182	51.6	9.8	0.110	89.0
2954	Kimball	9.4	0.147	64.0	10.7	0.097	110.3
4007	Cheyenne	9.3	0.187	49.7	11.0	0.128	86.0
2952	Kimball	8.5	0.147	57.8	8.1	0.086	94.2
4011	Deuel	8.3	0.147	56.5	11.4	0.101	112.9
2948	Kimball	7.8	0.137	56.9	8.2	0.081	101.2
9 samples, average.........		9.2	0.162	57.1	10.3	0.101	103.1
Probably error of a single variant............				±2.9	......	....	±9.9

TABLE 3—*Continued*

0-6-INCH SAMPLE NUMBER	COUNTY	0-6-INCH			7-12-INCH		
		Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
			per cent			*per cent*	
Rosebud loam							
2940	Kimball	10.7	0.176	60.8	11.3	0.120	94.2
4031	Chase	9.4	0.179	52.5	11.0	0.109	100.9
2942	Kimball	9.1	0.151	60.3	10.4	0.086	120.9
4037	Chase	8.6	0.142	60.5	10.5	0.102	102.9
3971	Kimball	8.0	0.147	54.4	11.3	0.116	97.4
3985	Kimball	7.8	0.150	52.0	8.9	0.083	107.2
3981	Kimball	7.5	0.143	52.4	8.4	0.081	103.6
3969	Kimball	7.4	0.154	48.0	8.8	0.108	81.5
4025	Chase	7.2	0.125	57.5	7.9	0.079	100.0
3977	Kimball	7.1	0.131	54.1	6.6	0.087	75.8
3653	Sioux	6.1	0.098	62.2	7.1	0.073	112.7
11 sample, average........		8.1	0.145	55.9	9.3	0.094	99.7
Probable error of a single variant.............				±3.1	......	....	±8.6
Holdrege silt loam—Western Nebraska							
2934	Lincoln	9.2	0.155	59.3	8.9	0.118	75.4
3454	Lincoln	8.6	0.179	48.0	8.4	0.116	72.4
A-Mc	Red Willow	8.6	0.162	53.0	10.0	0.107	93.4
2936	Lincoln	8.5	0.164	51.8	9.0	0.119	75.6
2938	Lincoln	8.4	0.149	56.4	8.1	0.105	77.1
A-Wa*	Chase	8.3	0.159	52.2	8.8	0.106	83.0
3456	Lincoln	8.1	0.150	54.0	7.0	0.083	84.4
7 samples, average.........		8.5	0.160	53.5	8.6	0.108	80.2
Probable error of a single variant.............				±2.4	......	....	±4.9
Wankesha silt loam							
2477	Boone	12.4	0.306	40.5	11.8	0.235	50.2
2972	Saunders	11.8	0.261	45.2	12.3	0.194	63.4
3736	Saunders	11.1	0.262	42.4	11.2	0.206	54.4
1385	Saunders	9.5	0.276	34.4	9.9	0.179	55.3
2501	Gage	9.0	0.256	35.1	8.7	0.172	50.5
1458	Washington	8.6	0.252	34.1	8.6	0.194	44.3
6 samples, average........		10.4	0.269	38.6	10.3	0.197	53.0
Probable error of a single variant.............				±3.2	......	....	±4.3

* Holdrege Loam

TABLE 3—*Continued*

0–6-INCH SAMPLE NUMBER	COUNTY	0–6-INCH			7–12-INCH		
		Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
			per cent			*per cent*	
Hall silt loam							
2646	Hall	9.2	0.235	39.1	8.5	0.133	63.9
2648	Hall	8.6	0.202	42.6	9.3	0.155	60.0
1302	Hall	8.2	0.198	41.4	7.9	0.125	63.2
1292	Hall	7.8	0.195	40.0	8.3	0.161	51.5
4 sample, average.........		8.5	0.208	40.8	8.5	0.144	59.7
Probable error of a single variant.............				±1.0	......	....	±3.9
Bridgeport very fine sandy loam							
3959	Scotts Bluff	8.1	0.120	67.5	8.5	0.100	85.0
3883	Scotts Bluff	7.4	0.119	62.2	7.7	0.078	98.7
3879	Morrill	7.1	0.115	61.7	7.6	0.104	73.1
3887	Scotts Bluff	6.8	0.101	67.3	7.0	0.075	93.2
4 samples, average.........		7.4	0.114	64.7	7.7	0.089	87.5
Probable error of a single variant.............				±2.1	......	....	±7.5
Valentine fine sand							
3855	McPherson	3.6	0.096	37.5	3.3	0.059	56.0
3851	McPherson	3.0	0.080	37.5	3.0	0.051	58.9
4263	Cherry	2.6	0.068	38.2	2.4	0.045	53.3
3862	Lincoln	2.3	0.057	40.4	1.9	0.033	57.6
4 samples, average.........		2.9	0.075	38.4	2.7	0.047	56.5
Probable error of a single variant.............				±0.9	......	....	±1.6
O'Neill sand							
2636	Hall	3.68	0.102	36.0	3.59	0.062	57.9
1284	Howard	3.17	0.076	41.7	2.04	0.043	47.4
2632	Hall	2.87	0.068	42.2	2.48	0.038	65.2
1286	Howard	2.70	0.079	34.2	1.26	0.026	48.4
4 samples, average.........		3.10	0.081	38.5	2.34	0.042	54.7
Probable error of a single variant.............				±2.7	......	....	±5.7

TABLE 3—*Continued*

0–6-INCH SAMPLE NUMBER	COUNTY	0–6-INCH			7–12-INCH		
		Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
			per cent			*per cent*	
		Knox silt loam					
2928	Douglas	12.1	0.199	60.8	12.1	0.143	84.6
2872	Washington	11.9	0.249	47.8	11.7	0.179	65.4
2890	Washington	10.6	0.188	56.4	10.8	0.219	49.3
2823	Dakota	10.4	0.235	44.2	9.3	0.131	71.0
1369	Burt	10.2	0.131	77.8	10.1	0.067	150.8
5 samples, average.........		11.0	0.200	57.4	10.8	0.148	84.2
Probable error of a single variant.............				±8.9	......	......	±26.4
		Colby very fine sandy loam					
3219	Adams	10.7	0.180	59.4	10.9	0.150	72.6
2884	Dawson	10.4	0.137	76.0	10.8	0.090	120.0
3253	Kearney	10.2	0.167	61.1	10.8	0.085	127.0
3246	Kearney	8.6	0.174	49.4	8.5	0.101	84.1
4 samples, average.........		10.0	0.165	61.5	10.3	0.107	100.9
Probable error of a single variant.............				±7.5	......	......	±17.9
		Wabash silt loam					
2570	Pawnee	14.2	0.257	55.3	10.0	0.199	50.2
3696	Pierce	13.2	0.320	41.3	13.4	0.219	61.2
2962	Butler	12.3	0.296	41.5	10.9	0.188	58.0
2556	Richardson	12.1	0.232	52.1	12.1	0.159	76.1
2968	Saunders	11.3	0.281	40.2	11.5	0.206	55.8
2844	Knox	10.9	0.302	36.1	10.0	0.216	46.3
6 samples, average.........		12.3	0.281	44.4	11.3	0.198	57.9
Probable error of a single variant.............				±5.3	......	....	±7.3

It is interesting to note that the uniformity in all of these types is on the same order as that observed by Alway and Trumbell (see p. 423) for individual samples in a single field.

The last three types in table 3, viz., Knox silt loam, Colby very fine sandy loam, and Wabash silt loam are exceptional. The HC/N ratios in both 0 to 6- and 7 to 12-inch sections are highly variable and the probable error values are large. One could not estimate to any reliable degree, the nitrogen content of a sample of any of these types from the hygroscopic coefficient. The Knox type occupies the highly rolling and bluff lands of eastern Nebraska. It is a soil in destructional stages on which erosion is outrunning organic-matter-producing processes. Colby very fine sandy loam is also an unstabilized soil, both physio-

TABLE 4

Homogeneity in nitrogen content of one Kansas, and two Minnesota soil types

0–6-INCH SAMPLE NUMBER	COUNTY	0–6-INCH			7–12-INCH		
		Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
			per cent			*per cent*	
Pratt loam—Central Kansas							
3620	Rice	10.4	0.218	47.7	9.1	0.145	62.7
3448	McPherson	10.3	0.199	51.7	11.5	0.144	79.9
3442	McPherson	9.6	0.199	48.2	13.4	0.139	96.5
3606	Sedgwick	9.3	0.210	44.3	10.9	0.162	67.3
3608	Sedgwick	9.1	0.203	44.8	10.2	0.162	63.0
3444	McPherson	9.1	0.196	46.4	9.4	0.145	64.8
3622	Rice	8.6	0.188	45.7	9.9	0.141	70.2
3446	McPherson	8.2	0.178	46.0	9.9	0.128	77.3
3604	Sedgwick	7.6	0.186	40.8	8.0	0.141	56.7
3624	Rice	7.8	0.169	46.1	11.7	0.128	91.4
10 samples, average........		9.0	0.195	46.2	10.4	0.143	73.0
Probable error of a single variant............				±1.9	......	....	±9.0
Hemsted silt loam—Minnesota							
4289	Dakota	11.8	0.375	31.5	9.4	0.223	42.1
4297	Dakota	11.7	0.359	32.6	10.2	0.249	41.0
4293	Dakota	11.7	0.355	33.0	10.2	0.239	42.7
4295	Dakota	11.5	0.350	32.8	9.3	0.210	44.3
4291	Dakota	11.4	0.357	32.0	9.0	0.212	42.5
5 samples, average.........		11.6	0.359	32.4	9.6	0.227	42.5
Probable error of a single variant.............				±0.4	....	....	±0.6
Clarion silt loam—Minnesota							
4301	Stevens	19.2	0.471	40.8	16.9	0.310	54.5
4307	Stevens	18.6	0.567	32.8	16.4	0.401	40.9
4303	Stevens	16.4	0.427	38.4	14.2	0.273	52.0
4305	Stevens	16.0	0.517	30.9	14.0	0.373	37.5
4309	Stevens	14.4	0.391	36.8	11.6	0.179	64.8
5 samples, average.........		16.9	0.475	35.9	14.6	0.307	49.9
Probable error of a single variant............				±2.8	......	....	±7.4

graphically and ecologically. In some sections wind, during comparatively recent times, has drifted this soil type into low hummocks, which are now sparcely covered with vegetation, and in other places water has denuded the virgin prairie of organic matter. In such soil types, homogeneity in nitrogen content is not to be expected.

Wabash silt loam and all other alluvial soils, are in general physiographically and ecologically unstabilized to some degree. They are frequently subjected to overflow and are variable in drainage conditions. It is not surprising therefore, that the nitrogen content and HC/N ratios in Wabash silt loam vary.

Table 4 shows the homogeneity in nitrogen content of 3 types from outside Nebraska. The 10 fields of Pratt loam (identified by correlation with soils of the Pratt series in the published survey of Reno County, Kansas) are representative of a rather extensive area in central Kansas, yet are as uniform in the HC/N ratio of the first 6 inches as the soil types of Nebraska, and only slightly less uniform in the second 6 inches. The 5 fields of Hemstead silt loam from a single township in Dakota County, Minnesota (identified by correlation with the Hemstead silt loam in Ramsey County), are unusually uniform in both texture and nitrogen content. Clarion silt loam from Stevens County, Minnesota, exhibits a considerable degree of heterogeneity, which was not unsuspected at the time of sampling, for virgin areas were extremely difficult to find and not entirely satisfactory. The data for these types serve to indicate the application to other regions of the HC/N ratio method of studying nitrogen distribution in soils.

RELATION OF NITROGEN CONTENT TO SERIES AND TYPE

In table 5 is shown the type averages taken from table 3, and corresponding data for all other Nebraska soil type samples represented in this study by less than 4 samples each. The samples are arranged according to series and types in conventional groups based on source material and topography. For each series, an average based on the number of samples in each type, is shown.

From the standpoint of percentage only, the Wabash series shows the highest, and the O'Neill and Valentine series the lowest, nitrogen content. The Wabash series is generally ranked as one of the most productive in eastern Nebraska, whereas the O'Neill and Valentine are probably two of the least productive soils.

Should percentage of nitrogen by itself be used as an approximate index of the productiveness of a soil, the silt loams of the Carrington, Marshall, Grundy, Waukesha, Wabash, and central Nebraska Holdrege series would all be classed as highly productive soils. Likewise the Knox and Hall silt loams, which contain almost identically the same nitrogen in both the first and second inch sections, would be classed together. The Knox soil, however, is notably deficient in nitrogen from the standpoint of crop production, whereas the Hall silt loam compares favorably with the Grundy, which is one of the better soils of the prairies.

TABLE 5

Relation of the nitrogen content of Nebraska soils to series and type

-6-INCH SAMPLE NUMBER	SERIES AND TYPE	COUNTY	0-6-INCH			7-12-INCH		
			Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
				per cent			*per cent*	
		Glacial						
	Carrington							
.....	silt loam	22 samples	11.5	0.262	44.2	12.5	0.199	63.1
	Shelby							
2560	loam	Richardson	9.4	0.258	36.4	9.9	0.185	53.5
2564	loam	Pawnee	7.8	0.212	36.8	9.5	0.132	71.2
2496	loam	Gage	7.4	0.187	39.6	7.5	0.124	60.4
.....	Average	....	8.2	0.219	37.6	9.0	0.147	61.9
		Loessial						
	Marshall							
.....	silt loam	16 samples	11.2	0.252	44.8	11.6	0.183	64.3
2682	loam	Hall	7.8	0.187	41.7	8.0	0.140	57.1
.....	*Weighted average*	*17 samples*	*11.0*	*0.248*	*42.2*	*11.4*	*0.172*	*63.3*
	Grundy							
.....	silt loam	10 samples	10.3	0.252	40.9	10.5	0.172	61.7
	Holdrege							
A-Ho	silt loam Cent. Nebr.	Phelps	9.9	0.231	42.8	10.2	0.139	73.4
3239	silt loam Cent. Nebr.	Kearney	9.7	0.205	47.3	9.9	0.184	53.8
3226	silt loam Cent. Nebr.	Kearney	8.8	0.191	46.0	9.7	0.138	70.2
.....	Average	....	9.5	0.209	45.4	9.9	0.154	65.8
.....	silt loam, West. Nebr.	7 samples	8.5	0.160	53.5	8.6	0.108	80.2
.....	*Weighted average*	*10 samples*	*8.8*	*0.175*	*51.1*	*9.0*	*0.122*	*75.9*
	Knox							
.....	silt loam	5 samples	11.0	0.200	57.4	10.8	0.148	84.2

TABLE 5—*Continued*

0-6-INCH SAMPLE NUMBER	SERIES AND TYPE	COUNTY	0-6-INCH			7-12-INCH		
			Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
				per cent			*per cent*	
	Loessial—Continued							
	Colby							
3812	silt loam	Sherman	10.8	0.166	65.1	11.9	0.109	109.1
2690	silt loam	Hall	10.8	0.147	73.5	10.7	0.063	170.0
3832	silt loam	Custer	8.7	0.142	61.2	9.5	0.100	95.0
.....	very fine sandy loam	4 samples	10.0	0.165	61.5	10.3	0.107	100.9
2686	sandy loam	Hall	8.2	0.155	52.9	9.2	0.094	97.9
.....	*Weighted average*	*8 samples*	*9.8*	*0.158*	*62.3*	*10.3*	*0.099*	*109.5*
	Residual							
	Rosebud							
.....	silt loam	9 samples	9.2	0.162	57.1	10.3	0.101	103.1
.....	loam	11 samples	8.1	0.145	55.9	9.3	0.094	99.7
.....	*Weighted average*	*20 samples*	*8.6*	*0.153*	*56.4*	*9.8*	*0.097*	*100.2*
	Dawes							
4023	loam	Perkins	10.7	0.205	52.2	10.4	0.130	80.0
4019	loam	Perkins	9.7	0.194	50.0	8.0	0.107	74.8
4013	silt loam	Deuel	8.8	0.179	49.2	9.8	0.108	89.9
.....	Average	3 samples	9.7	0.193	50.5	9.4	0.115	81.6
.....	*Valentine*	4 samples	2.9	0.075	38.4	2.7	0.047	56.5
	Terrace							
	Waukesha							
.....	silt loam	6 samples	10.4	0.269	38.6	10.3	0.197	53.0
	Hall							
.....	silt loam	4 samples	8.5	0.208	40.8	8.5	0.144	59.7
2669	very fine sandy loam	Hall	8.7	0.206	42.2	8.5	0.146	58.2
2665	very fine sandy loam	Hall	8.0	0.194	41.2	9.2	0.101	91.1
....	Average	....	8.4	0.200	41.7	8.9	0.124	74.7

TABLE 5—*Continued*

0-6-INCH SAMPLE NUMBER	SERIES AND TYPE	COUNTY	0-6-INCH			7-12-INCH		
			Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
				per cent			*per cent*	
	Terrace—Continued							
	Hall—Continued							
2661	fine sandy loam	Hall	7.2	0.175	41.2	6.6	0.134	49.2
2656	fine sandy loam	Hall	3.6	0.118	30.5	2.6	0.062	42.0
.....	Average	....	5.4	0.147	35.9	4.6	0.098	45.6
2673	sandy loam	Hall	4.7	0.131	35.9	3.4	0.059	57.6
.....	*Weighted average*	*9 samples*	*7.3*	*0.184*	*39.3*	*7.1*	*0.120*	*59.6*
	O'Neill							
2623	loam	Hall	7.4	0.188	39.4	6.4	0.090	71.1
2628	loam	Hall	6.6	0.169	39.0	5.6	0.100	56.0
....	Average	....	7.0	0.179	39.2	6.0	0.095	63.6
....	sand	4 samples	3.1	0.081	38.5	2.3	0.042	54.7
.....	*Weighted average*	*6 samples*	*4.4*	*0.114*	*38.7*	*3.6*	*0.060*	*57.7*
	Tripp							
3987	very fine sandy loam	Kimball	8.8	0.158	55.6	8.4	0.110	76.4
	Bridgeport							
.....	very fine sandy loam	4 samples	7.4	0.114	64.7	7.7	0.089	87.5
	Alluvial							
	Wabash							
2911	clay	Washington	19.4	0.302	64.2	18.9	0.207	91.4
2904	clay	Dodge	16.6	0.345	48.2	15.4	0.211	73.0
....	Average	....	18.0	0.324	56.2	17.2	0.209	82.2
2970	silty clay	Dodge	15.4	0.327	47.1	17.3	0.188	92.1
2907	silty clay	Dodge	14.7	0.355	41.4	14.9	0.208	71.6

TABLE 5—*Continued*

0-6-INCH SAMPLE NUMBER	SERIES AND TYPE	COUNTY	0-6-INCH			7-12-INCH		
			Hygroscopic coefficient	Nitrogen	HC/N	Hygroscopic coefficient	Nitrogen	HC/N
				per cent			*per cent*	
	Alluvial—Continued							
	Wabash—Continued							
.....	Average	....	15.1	0.341	44.3	16.1	0.198	81.9
2909	silty clay loam	Washington	14.5	0.335	43.3	13.7	0.220	62.3
.....	silt loam	6 samples	12.3	0.281	44.4	11.3	0.198	57.9
.....	*Weighted average*	*11 samples*	*14.1*	*0.305*	*46.4*	*13.5*	*0.202*	*67.1*
	Cass							
2920	silt loam	Douglas	17.1	0.574	29.8	14.8	0.356	41.6
2916	clay	Washington	16.9	0.281	60.1	19.2	0.205	93.6
2918	loam	Douglas	11.0	0.231	47.6	9.6	0.184	52.2
2677	sandy loam	Hall	6.4	0.183	35.0	5.0	0.098	51.0
1308	fine sandy loam	Hall	5.8	0.181	32.0	6.7	0.171	39.2
2922	fine sandy loam	Douglas	5.4	0.178	30.3	3.5	0.106	33.0
.....	Average	....	5.6	0.180	31.2	5.1	0.139	36.1
.....	*Weighted average*	*6 samples*	*10.4*	*0.271*	*39.1*	*9.8*	*0.187*	*51.8*
	Lamoure							
1316	clay loam	Hall	10.0	0.203	49.3	9.8	0.099	98.9
2641	fine sandy loam	Hall	8.2	0.212	38.7	7.4	0.111	66.6
.....	Average	2 samples	9.1	0.208	44.0	8.6	0.105	82.8

Percentage nitrogen alone is not a proper criterion of type differences. Texture must be taken into consideration. When this is done, that is, when the data are interpreted in the light of HC/N ratios, the soils of the Wabash series are seen to be highest of all in nitrogen because they happen to be heaviest in texture, and the soils of the Valentine and O'Neill series are lowest in nitrogen because they are the lightest in texture. On the same basis the Knox type is revealed as one deficient in nitrogen, whereas the Hall silt loam is as well filled with nitrogen as the highly productive Grundy soils.

The types and series may be classified into three groups: (*a*) soils with practically constant HC/N ratios, (*b*) soils with generally high but variable HC/N ratios, (*c*) soils with generally low but variable HC/N ratios. The Carrington, Shelby, Marshall, Grundy, Holdrege, Rosebud, and the terrace

TABLE 6

Relative nitrogen content of the second 6-inch section of Carrington silt loam expressed in percentage of that found in the first 6-inch section

0–6-INCH SAMPLE NUMBER	$\frac{\text{HC (7–12-INCH)}}{\text{HC (0–6-INCH)}}$	$\frac{\text{N (7–12-INCH)}}{\text{N (0–6-INCH)}}$	
		Direct	Corrected for texture
	per cent	*per cent*	*per cent*
2743	101.3	81.1	80.0
3400	109.3	74.0	67.5
2771	111.1	73.5	66.2
2736	108.0	78.3	72.3
2934a	108.1	73.1	67.6
2491	109.1	77.7	71.2
2617	108.3	79.9	73.9
2764	110.8	75.2	67.9
1139.1	106.9	73.8	69.1
2715	102.5	81.5	79.7
2785	97.5	74.4	76.4
1381	110.4	71.9	65.1
2757	135.1	79.7	59.4
A-L	111.5	70.9	63.6
2528	100.0	72.7	72.7
2856	105.3	78.5	74.3
2574	110.7	71.7	64.6
A-WW	109.8	75.2	69.8
1373	103.6	72.0	69.4
2535	111.1	92.0	82.7
1141.1	106.0	68.9	64.8
1140.1	118.3	81.0	68.3
Average...........	108.8	76.2	70.3
Probable error of a single variant.........		±4.0	±3.9

soils fall in the first group, the Knox and Colby soils in the second group, and the alluvial soils in the third group. The first group may be called "stabilized," and the other two groups "unstabilized" soils.[4]

No series of stabilized soils is represented by sufficient samples of a variety of well defined types to allow the statement that the HC/N ratio is as reasonably constant for the series as it is for the types, but there are indications in the Hall and O'Neill series that such constancy can be expected.

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[4] Plant ecologists use the term "stabilization" for that process of adjustment of vegetation to environment which eventuates in the highest degree of permanence in the plant cover, and "stabilized" and "unstabilized" as descriptive of the degree of adjustment. It seems to the authors that the same words may well be used with reference to soil formations, particularly in respect to equilibria between vegetation and organic matter. The term "stabilized" as applied to a soil type is practically synonymous with the term "mature." The term "unstabilized," however, would include all other soils, recent, young, youthful, and immature, as well as aged or degraded soil. (See report of committee on Soil Terminology, American Soil Survey Association, Bul. 8, p. 74, 1927.) The authors prefer not to use the terms "mature," etc., in connection with this paper as these terms presuppose recognition of profile characteristics which the authors have not found necessary to take into consideration.

Within the stabilized group of soils there are some series differences. The Waukesha, Hall, O'Neill, and Valentine soils have similar, low HC/N values in both the 0 to 6-inch and 7 to 12-inch sections, and the HC/N ratios for Grundy silt loam are but slightly higher. The Carrington and Marshall soils are strikingly alike in HC/N ratios, but the values are somewhat higher than for the types above. The Holdrege soils of central and western Nebraska are unlike enough in HC/N values to belong to different series. The Rosebud and Bridgeport soils differ from the others in having high but fairly constant HC/N ratios. The Dawes series, which is found in the same region as the Rosebud soils, differs from the latter in having lower HC/N ratios, that is, a higher nitrogen content in both first and second 6-inch sections.

Within the unstabilized groups of soils, type differences are overshadowed by type heterogeneity. Nevertheless the Knox soils contain more nitrogen than the Colby, and the Cass soils more nitrogen than the Wabash on an equivalent texture basis.

The homogeneity of the soil type with respect to the distribution of nitrogen between the first and second 6-inch sections is shown for Carrington silt loam in table 6. Since the hygroscopic coefficient of the second 6 inches of this soil is higher in practically every case, on the average 8.8 per cent higher, than that of the first 6 inches, it would be logical to expect the proportion of nitrogen to be higher also. Hence the distribution of nitrogen is shown in two ways, by direct calculation (column 3), and corrected for texture (column 4). The latter is, of course, simply the HC/N ratio of the 7 to 12-inch section divided by the HC/N ratio of the 0 to 6-inch section.

The same degree of homogeniety of the type is exhibited in respect to the distribution of nitrogen between the first and second 6 inches as is exhibited by the type itself in nitrogen content. On the average the second 6 inches of Carrington silt loam contains 76.2 per cent as much actual nitrogen as the first 6 inches. The chances are even that this value will be found for any case, within ±4.0 per cent. Knowing the nitrogen content of the 0 to 6-inch section of Carrington silt loam, one could estimate the nitrogen content of the 7 to 12-inch section within ± 0.010 per cent in 50 out of 100 cases. At the widest the error in calculated nitrogen would be only 0.041 per cent. When corrected for texture, the nitrogen content of the second 6-inch section is 70.3 per cent of that in the first 6-inch section. The probable error of any single variant is practically the same as by the direct method of calculation.

Table 7 shows the ratio of the actual nitrogen in the 7 to 12-inch section to that in the 0 to 6-inch section expressed as percentage for various Nebraska soil types. The variability in nitrogen distribution between the first and second 6 inches appears to be even less among types, than among fields within a typical stabilized type, such as is illustrated in table 7. On the average, the second 6 inches of virgin prairie soil contains two-thirds as much nitrogen as the first 6 inches. A few types, particularly those of the O'Neill, and Lamoure series, seem to be characterized by having in the second 6 inches a relatively low content of nitrogen as compared with the first 6 inches. Knox silt loam, an outstanding unstabilized soil type usually characterized by its shallow surface soil, carries proportionally as much of its total surface-foot nitrogen in the second 6 inches as does Marshall or Carrington silt loams, both of which are noted for their deep surface soils.

TABLE 7

Relative nitrogen content of the second 6-inch section of Nebraska soil type expressed as percentage of that found in the first 6-inch section

SERIES AND TYPE	DESCRIPTION	NUMBER OF SAMPLES	$\frac{\text{7–12-INCH N}}{\text{0–6-INCH N}} \times 100$
Eastern and Central Nebraska			
Carrington silt loam	Glacial upland	22	76.2
Knox silt loam	Unstabilized loessial upland	5	74.0
Holdrege silt loam	Loess plains	3	73.7
Waukesha silt loam	Terrace	6	73.2
Marshall silt loam	Stabilized loessial upland	16	72.6
Cass types	Unstabilized bottom land	6	69.0
Grundy silt loam	Loess plains	10	68.3
Shelby loam	Glacial upland	3	67.1
Wabash types	Unstabilized bottom land	11	66.7
Hall types	Terrace	9	65.2
Valentine fine sand	Sand hills	4	62.7
Colby types	Unstabilized loessial upland	8	62.6
O'Neill types	Terrace	6	52.6
Lamoure types	Unstabilized bottom land	2	50.0
Average		..	66.7
Average 9 stabilized types		..	68.0
Average 5 unstabilized types		..	64.5
Western Nebraska			
Bridgeport very fine sandy loam	Terrace	4	78.0
Tripp very fine sandy loam	Terrace	1	69.7
Holdrege silt loam	Loess plains	7	67.5
Rosebud types	Residual upland	20	63.4
Dawes loam	Residual upland	3	59.6
Average		..	67.6
Average of all		..	66.9

Some of the western Nebraska soils, like the Bridgeport and Tripp, commonly thought of as having thin surface layers seem to carry an unexpectedly high proportion of their nitrogen in the second 6 inches. The three samples of the Dawes, a series which is considered to have a deep surface layer, actually carry a slightly lower proportion of nitrogen in the second 6 inches than does the average Rosebud soil commonly thought of as having a shallow surface layer.

In the 147 pairs of samples shown in tables 3 and 5 only one case occurs where the nitrogen content of the second 6-inch section exceeds that of the first. This is in Knox silt loam which has been described as possessing destructional features. In the experience of the authors (14) with virgin prairie soils a higher nitrogen content is never found in a lower layer than in layers above, except in cases of buried soils or where silting by wind or water has occurred comparatively recently.

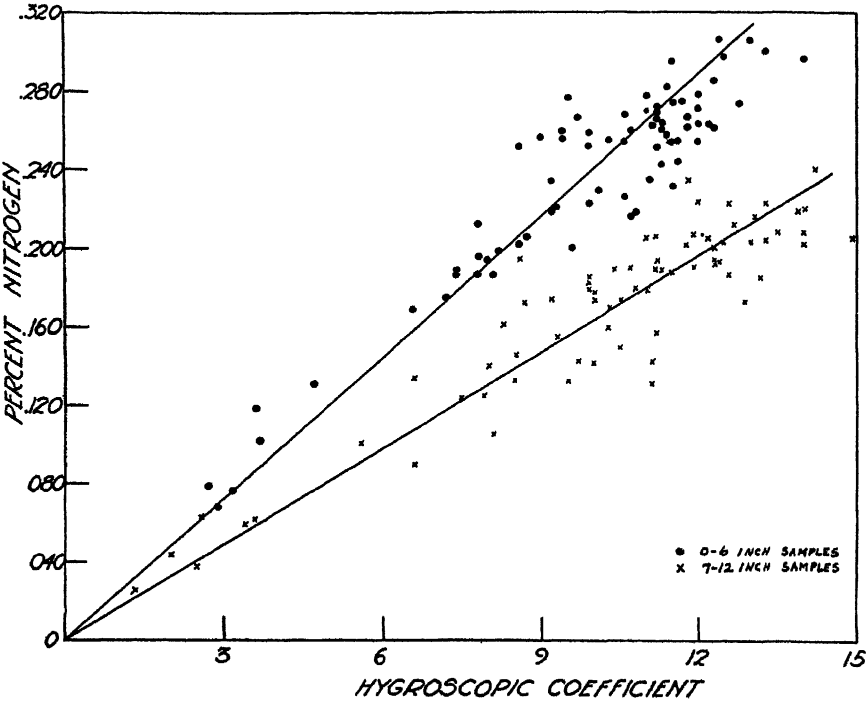

FIG. 2. THE RELATION OF NITROGEN CONTENT TO HYGROSCOPIC COEFFICIENT (TEXTURE) FOR 73 SETS OF 0 TO 6-INCH AND 7 TO 12-INCH SAMPLES REPRESENTING 7 COMPARABLE EASTERN NEBRASKA SOIL SERIES

FACTORS AFFECTING NITROGEN CONTENT

Texture

The relation of nitrogen content to texture has already been briefly discussed, and the assumption has been made that the relation to the hygroscopic

TABLE 8

Relation of nitrogen content to texture in stabilized virgin eastern Nebraska upland and terrace soil types

SERIES	CLASS	NUMBER OF SAMPLES	HYGROSCOPIC COEFFICIENT	NITROGEN	HC/N
				per cent	
		0–6-inch sections			
Carrington*	Silt loam	11	12.2	0.272	45.1
Marshall*	Silt loam	8	11.9	0.261	45.6
Carrington†	Silt loam	11	10.8	0.251	43.2
Marshall†	Silt loam	8	10.6	0.243	43.9
Waukesha‡	Silt loam	6	10.4	0.269	38.6
Grundy	Silt loam	10	10.3	0.252	40.9
Hall‡	Silt loam	4	8.5	0.208	40.8
Hall‡	Very fine sandy loam	2	8.4	0.200	41.7
Shelby	Loam	3	8.2	0.219	37.6
Marshall	Loam	1	7.8	0.187	41.7
O'Neill‡	Loam	2	7.0	0.179	39.2
Hall‡	Fine sandy loam	2	5.4	0.147	35.9
Hall‡	Sandy loam	1	4.7	0.131	35.9
O'Neill‡	Sand	4	3.1	0.081	38.5
Average		73	....	......	40.6
		7–12-inch sections			
Carrington*	Silt loam	11	13.5	0.209	65.0
Marshall*	Silt loam	8	12.7	0.205	62.1
Carrington†	Silt loam	11	11.6	0.189	61.2
Marshall†	Silt loam	8	10.5	0.161	86.5
Waukesha‡	Silt loam	6	10.3	0.197	53.0
Grundy	Silt loam	10	10.5	0.172	61.7
Shelby	Loam	3	9.0	0.147	61.9
Hall‡	Very fine sandy laom	2	8.9	0.124	74.7
Hall‡	Silt loam	4	8.5	0.144	59.7
Marshall	Loam	1	8.0	0.140	57.1
O'Neill‡	Loam	2	6.0	0.095	63.6
Hall‡	Sandy loam	1	4.7	0.059	58.3
Hall‡	Fine sandy loam	2	4.6	0.098	45.6
O'Neill‡	Sand	4	2.3	0.042	54.7
Average		73	....	......	60.4

* Heavy-textured half of total.

† Light-textured half of total.

‡ Terrace types.

coefficient is directly linear to a degree that permits of a correction of textual heterogeneity in the soil type, and interpretation of homogeneity in nitrogen by means of HC/N ratios. The data presented in tables 3 and 4 seem to indicate that this assumption is not unfounded or useless. Other factors in addition to texture affect nitrogen content; therefore, in an analysis of the strict validity of the above assumption to a wide range of textures it is necessary to limit one's study either to soils of a single series or to several series which are comparable climatically. Furthermore the soils must be of stabilized characteristics. As no single series of soils is represented in this study by a wide enough range of textures, 7 series have been selected and the nitrogen contents of the 73 sets of samples plotted against their hygroscopic coefficients as shown in figure 2. The series and types selected are shown in table 8. They are all stabilized soils from eastern Nebraska. To obtain sufficient variety in texture it has been necessary to include both upland and terrace soils. The fact that level plains and terrace soils are somewhat richer in nitrogen than rolling upland soils will confuse only slightly the texture-nitrogen correlation in either group.

The linear relation of nitrogen to hygroscopic coefficient in both the first and second 6-inch sections is obvious. The coefficients of correlation of nitrogen to hygroscopic coefficient for the entire set of seventy-three 0 to 6- and 7 to 12-inch samples are 0.710 ±0.039 and 0.690 ±0.041, respectively. For the two types, Carrington and Marshall silt loams, which are represented by adequate numbers for calculation the coefficients of correlation are 0.747 ±0.068 and 0.633 ±0.101, respectively, for the 0 to 6-inch, and 0.647 ±0.084 and 0.645 ±0.098 for the 7 to 12-inch sections.

Rainfall

In an analysis of the correlation of nitrogen content with rainfall it is obviously necessary to limit comparison to stabilized soils of comparable physiographic expression and identical texture. In Nebraska, soil texture is generally somewhat coarser in the drier western part of the state, and from the list of samples in this study it is not possible to select any considerable number possessing corresponding hygroscopic coefficients. In 5 comparable upland series, however, 76 sets of samples are classified as loams or silt loams and correction for texture seems allowable. Of these 5 series the Grundy, Holdrege and Rosebud occur in large, fairly uniform areas in sequence from east to west over a comparatively level plain, the rainfall decreasing uniformly from 30 to 16 inches. The Marshall and Carrington occupy a rolling plain to the east of the main body of the Grundy, the rainfall of this section varying from 28 to 32 inches.

In table 9 are shown the average hygroscopic coefficients, nitrogen contents, and HC/N ratios for these soils classified into four groups, which the distribution of the various samples (fig. 1) appears to permit. With respect to numbers of samples, the eastern and western groups are well represented. The samples of Holdrege silt loam fall logically into two groups, the numbers in each appearing small. However, in the central group occurs one, and in the west central group two of the Alway-McDole samples, which as previously explained are composites of 5 fields each, hence the data for these groups are probably more reliable than they appear. The annual precipitation for each type and group has been obtained by averaging for each field sampled the mean annual precipitation at the nearest long-time weather station. The average data for the different groups have been calculated by weighting each type for the number of samples contained, and the average nitrogen contents of each group have been reduced to a comparable texture basis of a hygroscopic coefficient of

TABLE 9

The relation of the nitrogen content of stabilized upland virgin Nebraska soils to rainfall

NUMBER OF FIELDS SAMPLED	TYPE	PRECIPITATION		0–6-INCH			7–12-INCH			AVERAGE 0–12-INCH	
		Annual	Relative	HC	N	HC/N	HC	N	HC/N	Per cent	Relative
		inches			*per cent*			*per cent*			
				Eastern							
16	Marshall silt loam	30.4	..	11.2	0.252	44.8	11.6	0.183	64.2	0.218	98
22	Carrington silt loam	30.3	..	11.5	0.262	44.2	12.5	0.199	63.1	0.231	104
10	Grundy silt loam	28.8	..	10.3	0.252	40.9	10.5	0.172	61.7	0.212	95
48	Weighted average	30.2	100	11.0	0.256	43.8	11.8	0.188	63.2	0.222	100
N content on basis HC = 10......		..		..	0.233	..	..	0.159	...	0.196	100
				Central							
3	Holdrege silt loam	23.8	79	9.5	0.209	45.4	9.9	0.154	65.8	0.182	82
N content on basis HC = 10......		..		..	0.220	...	..	0.156	...	0.188	96
				West Central							
7	Holdrege silt loam	19.0	63	8.5	0.160	53.5	8.6	0.108	80.2	0.134	60
N content on basis HC = 10......		..		..	0.188	..	..	0.126	...	0.157	80
				Western							
9	Rosebud silt loam	15.9		9.2	0.162	57.1	10.3	0.101	103.1	0.132	59
11	Rosebud loam	16.7		8.1	0.145	55.9	9.3	0.094	99.7	0.120	54
20	Weighted average	16.4	54	8.6	0.153	56.4	9.8	0.097	100.2	0.125	56
N content on basis HC = 10......		..		..	0.178	..	..	0.099	...	0.139	71

10. The last two columns of the table show the average and the relative nitrogen content of the first foot of soil, using eastern Nebraska as 100.

The actual nitrogen content of the first foot is in very close proportion to the average precipitation of each group, but when correction is made for texture, western Nebraska soils appear to be relatively richer, and simple proportionality to rainfall disappears. Figure 3 shows the apparent relation of nitrogen content to precipitation on an equivalent texture basis.

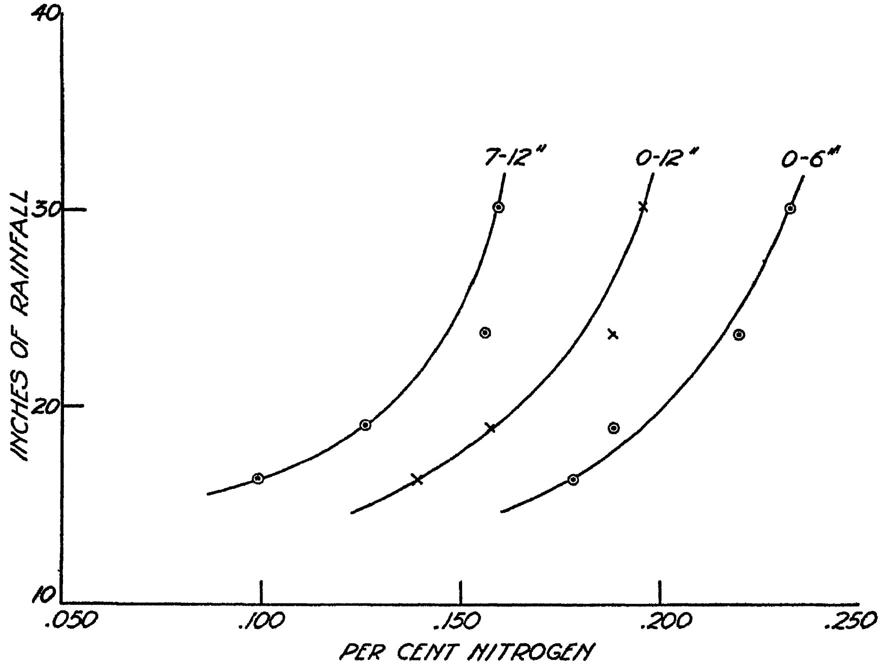

Fig. 3. Relation of the Nitrogen Content of Stabilized Upland Virgin Nebraska Soil Types to Mean Annual Precipitation

Each datum shown on the graph is an average of several samples reduced to an equivalent texture basis of a hygroscopic coefficient of 10.

Although the four groups do not by any means furnish enough data to establish the exact graphical character of the relation of nitrogen content to rainfall, it is apparently a curve.

EFFECT OF TOPOGRAPHIC EXPRESSION ON NITROGEN CONTENT

In table 5 it has been observed that soil types and series differ widely in nitrogen content when the latter is corrected for texture. Certain soil types had low HC/N ratios and others had high HC/N ratios. The data in table 9 indicate that in part these differences are due to rainfall. In table 10 data for climatically comparable soils taken from preceding tables are assembled to show the effect of topography on nitrogen content. Only the HC/N ratios and probable errors of single ratios are shown.

Upland soils and flat terraces have fairly constant and low HC/N ratios; stabilized rolling upland soils have constant but somewhat higher HC/N ratios. Very rolling and broken upland soils have high and variable HC/N ratios, particularly in the second 6-inch section. Flat bottom lands on the average have low but variable HC/N ratios. The common impression that bottom soils are among the most fertile soils is not substantiated, as far as nitrogen is concerned, by the data shown in table 10, for the two series Wabash

TABLE 10

The effect of topographic expression on nitrogen content as indicated by hygroscopic coefficient-nitrogen ratios and probable error, in climatically comparable eastern Nebraska soils

SOIL TYPES	NUMBER SAMPLES	0–6-INCH SECTION		7–12-INCH SECTION	
		HC/N	± Pe*	HC/N	± Pe*
Flat terraces					
Waukesha silt loam	6	38.6	3.2	53.0	4.3
O'Neill sand	4	38.5	2.7	54.7	5.7
Hall silt loam	4	40.8	1.0	59.7	3.9
Average	..	39.3	2.3	55.8	4.6
Flat upland					
Grundy silt loam	10	40.9	2.3	61.7	5.5
Rolling upland					
Carrington silt loam	22	44.2	1.9	63.1	3.4
Marshall silt loam	16	44.8	2.1	64.3	5.9
Average	...	44.5	2.0	63.7	4.7
Broken land					
Knox silt loam	5	57.4	8.9	84.2	26.4
Colby types	8	62.3	6.2	109.5	20.4
Average	..	59.9	7.6	96.9	23.4
Flat bottom land					
Wabash types	11	46.9	5.5	67.7	10.3
Cass types	6	39.1	8.2	51.8	14.6
Average	..	43.0	7.4	59.8	12.5

* ± Pe = Probable error of the HC/N ratio of a single sample.

and Cass have no more nitrogen in proportion to texture than flat terrace and upland soils.

SUMMARY AND DISCUSSION

Texture is the outstanding factor determining nitrogen content in any soil type, and all soil types exhibit considerable variation in texture, therefore, they exhibit a corresponding variation in nitrogen content. Textural variations may be expressed numerically in terms of the hygroscopic coefficient, and a calculation of the ratio of hygroscopic coefficient to nitrogen provides a means of eliminating the textural effect on the content of the latter in virgin prairie soils, and hence makes possible a study of the distribution of nitrogen in various series and types.

When this method of study is employed, some soil types are found to be so homogenous in nitrogen content that, given the hygroscopic coefficient and the depth to which a sample was taken, the nitrogen content may be calculated mathematically to a fair degree of accuracy. Other soil types are decidedly heterogenous. The homogenous types appear to be those which are developed on gently rolling or nearly flat uplands and terraces. The heterogenous types appear to be those with destructional or constructional features, and are found in broken lands or on flood plains.

Nitrogen homogeneity is to be found in both the first and second 6-inch sections of soil. At the same time there may be a corresponding degree of homogeneity in the relative distribution of nitrogen between these two sections.

In a series of homogenous types, nitrogen content varies with the rainfall and with topography, level types containing nore nitrogen than rolling types under the same precipitation, probably because of a difference in the effectiveness of rainfall in such cases.

The relative distribution of nitrogen between the first and second 6-inch soil sections differs slightly among the types, but not enough to warrant the conclusion that this feature is an outstanding type characteristic in any case.

The conclusions above may be applied to organic matter as well as to nitrogen, for the two constituents bear a fairly constant ratio to each other both in extremes of rainfall and of texture. It must be stressed that these conclusions have been based on representative samples of Nebraska types, and apply only to virgin grassland soils. It is believed, however, that they may apply in some degree to other sections of the grassland region.

It is pertinent to inquire concerning the application of these conclusions to soil classification and mapping. If homogeneity in organic matter and nitrogen is a desideratum of the soil type, then the most important thing of all to stress is textural uniformity; however, the surveyor has probably already approached the practical limit along this line.

The second point to stress probably is rainfall. Fortunately rainfall alters other properties of the soil profile in addition to the manifestations of organic matter, and with a better understanding of the way in which these properties are affected should come more accurate series distinction with respect to their organic matter differences. Soil color may not be the best criterion of the rainfall factor, although in the distinguishing of major soil regions, such as Marbut (10) has attempted in the Great Plains, it is probably an adequate aid. The actual determination of HC/N ratios would seem to be a good way of accurately defining the existence of such broad soil regions, and even of transitional series.

From the organic matter standpoint, the distinctions of series on the basis of topography, such as have been made in the past by all soil surveyors, seem to be well justified. Actual differences in organic matter content seem to exist between soils developed on rolling lands, and on level upland plains, or on terraces and on flood plains. Flood plain types are apparently heterogenous, whereas terrace soils are relatively uniform. In cases of dispute as to whether a type should be classified as terrace or bottom, the matter could well be settled in the laboratory by means of HC/N ratios. In some cases of differentiation of series in rolling uplands, the same test might apply, for some upland soils like the Knox silt loam are apparently characterized as definitely by their heterogeneity as others like the Marshall silt loam of southeastern Nebraska are characterized by their homogeneity.

The data presented in this paper are not sufficient to support discussion of the importance of soil origin or profile features in their relation to organic matter content. The similarity in nitrogen content of the loessial Marshall to the adjacent glacial Carrington silt loam, or of the level loessial Grundy to the terrace Hall and Waukesha silt loams has been noted. In spite of their differences in origin the two former and the three latter might well be combined into two types as far as their organic matter contents alone are concerned. Actually the Marshall and Carrington silt loams of southeastern Nebraska are very similar in profile features, as are also the Grundy and the Hall silt loam of this study. It would be interesting to examine data from a larger number of series representing a wider range of source material in order to observe what features or origin or profile, if any, are as important as topography and rainfall.

REFERENCES

(1) Alway, F. J. 1916 Report of the committee on soil classification and mapping. *Jour. Amer. Soc. Agron.* 8: 387–390.

(2) Alway, F. J., Klein, M. A., and McDole, G. R. 1917 Some notes on the direct determination of the hygroscopic coefficient. *Jour. Agr. Res.* 11: 147–166.

(3) Alway, F. J., and McDole, G. R. 1916 The loess soils of the Nebraska portion of the Transition Region: I. Hygroscopicity, nitrogen, and organic carbon. *Soil Sci.* 1: 197–238.

(4) Alway, F. J., and Trumbell, R. S. 1912 On the sampling of prairie soils. *Nebr. Agr. Exp. Sta. Ann. Rept.* 1912: 35–51.

(5) Alway, F. J., and Vail, C. E. 1912 The relative amounts of nitrogen, carbon, and humus in some Nebraska soils. *Nebr. Agr. Exp. Sta. Ann. Rept.* 1912: 145–168.

(6) Blair, A. W., and McLean, H. C. 1921 The chemical composition of the soils of the Camden area of New Jersey. N. J. Agr. Exp. Sta. Bul. 346.

(7) Brown, P. E., and O'Neal, A. M. 1923 The color of soils in relation to organic matter content. Iowa Agr. Exp. Sta. Res. Bul. 75.

(8) De Turk, E. E. 1922 The relation between soil type and chemical composition. *Rept. 3rd Ann. Meet. Amer. Assoc. Soil Survey Workers Bul.* 4 (v. 2): 130–149.

(9) Fraps, G. S. 1922 Organic constituents of the soil. Tex. Agr. Exp. Sta. Bul. 300.

(10) Marbut, C. F. 1923 Soils of the Great Plains. *Ann. Assoc. Amer. Geogr.* 13 (2): 41–66.

(11) Pendleton, R. L. 1919 Are soils mapped under a given type name by the Bureau of Soils method closely similar to one another? *Cal. Univ. Pubs. Agr. Sci.* 3: 369–498.

(12) Read, J. W., and Ridgell, R. H. 1922 On the use of the conventional carbon factor in estimating soil organic matter. *Soil Sci* 13: 1–6.

(13) Russel, J. C. 1918 Relation of certain physical and chemical properties to the soil class. (Unpub. Master's Thesis, Univ. Minn.).

(14) Russel, J. C., and Engle, E. B. 1925 Soil horizons in the central prairies. *Rept. 5th Ann. Meet. Amer. Soil Survey Assoc. Bul.* 6 (v. 1) 1–18.

(15) Sievers, F. J., and Holtz, H. F. 1926 The significance of nitrogen in soil organic matter relationships. Wash. Agr. Exp. Sta. Bul. 206.

(16) Stump, L. A. 1918 The relation of certain physical constants, and nitrogen to soil class in the case of Reno County, Kansas soils. (Unpubl. Master's Thesis, McPherson Col.).

(17) Walker, S. S. 1920 Chemical composition of some Louisiana soils as to series and texture. La. Agr. Exp. Sta. Bul. 177.

THE QUINHYDRONE ELECTRODE AND THE SOIL REACTION

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The application of the quinhydrone electrode to soil investigations was made by Biilmann (4) and by Christensen and Tovborg-Jensen (10) in 1924. This method for the determination of pH in soils proved to have several advantages over the hydrogen electrode as well as over the colorimetric method; for example, in the first case the necessity for coating the electrodes with platinum black is removed; and in the second, turbidity in solutions is no longer a factor. Because of the speed with which determinations can be made, due to the quickness of the quinhydrone electrode to attain equilibrium, and to the fact that it is not "poisoned" by soils or affected by nitrates (6, 8), it is rapidly coming into general use. Further investigations on methods and accuracy are therefore desirable.

The theory of the quinhydrone electrode has been given by Biilmann (2, 3, 6), LaMer and Parsons (22), and by Kolthoff and Furman (21). The solid quinhydrone which is added to the soil-water mixture, dissolves and dissociates into equi-molecular quantities of hydroquinone and quinone. From the equation:

$$\pi \text{ (E.M.F.)} = \frac{RT}{2F} \, ln \, \frac{\text{(quinone)}}{\text{(hydroquinone)}} + \frac{RT}{F} \, ln \, (\text{H}^+) + \text{K} \ldots\ldots\ldots\ldots \text{(A)}$$

the first term is zero when the concentration of quinone is the same as the concentration of hydroquinone, and therefore

$$\pi = \frac{RT}{F} \, ln \, (\text{H}^+) + \text{K} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(B)}$$

If the activity of the ions is disregarded (21, p. 1) K is π (or π_o) when the concentration of the hydrogen ion is 1.

Biilmann (2), by checking the quinhydrone electrode against a hydrogen electrode in solutions of the same H^+ concentration, found that at 25°C. π_o = 0.6990 and at 18°C., 0.7044, the platinum of the quinhydrone electrode being positive to the hydrogen electrode. Thus at 18°

$$\pi = 0.0577 \log (\text{H}^+) + 0.7044 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(C)}$$

and at 25°

$$\pi = 0.0591 \log (\text{H}^+) + 0.6990 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(D)}$$

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[1] Contribution from the Department of Chemistry.

When the quinhydrone electrode has been used in soil determinations, the second half cell has been either one of the calomel electrodes or a special half cell of 0.01 *N* HCl and 0.09 *N* KCl, introduced by Veibel (27). The pH of this mixture is 2.03 (6), or of a similar mixture of 0.01 *N* HCl and 0.09 *N* NaCl, 2.038 (25, p. 24). Biilmann and Tovborg-Jensen prefer this electrode to the calomel half cell because it is easily made up and has a comparatively high pH value. It has the drawback, however, that a fresh one must be made each day. The equation for this (6) is

$$\text{pH} = 2.03 + \frac{\pi}{0.0001984T} \text{.........................(E)}$$

Veibel compared it with the normal and 3.5 normal calomel half cells for the amount of variation, and found that it could be reproduced without greater change than the calomel cells, but that it was not markedly more accurate.

Hissink and Van der Spek (19) use the normal calomel electrode as the half cell, whereas Baver (1) favors the saturated calomel cell. Both of these have the advantage over Veibel's cell, that once made up, they will not vary for a considerable time. It is necessary, however, to reverse the poles of electrodes for some readings, e.g., with the normal calomel electrode as the half cell, at 18°. With the half cell instead of the normal hydrogen electrode the E.M.F. is 0.2864 too low (11, p. 456), and equation (C) becomes:

$$\pi + 0.2864 = 0.0577 \log (\text{H}^+) + 0.7044 \text{ ....(F)}$$

or $\text{pH} = 7.24 - \frac{\pi}{0.0577}$. If the pH is greater than 7.24 the E.M.F. is negative; if the pH is less than 7.24, the E.M.F. is positive. It is, however, a simple matter to place a double switch in the circuit and, when necessary, reverse the poles without touching the electrodes. Veibel's electrode has the advantage that the change would occur at 2.03, so that for the soil work the potential would rarely alter its sign.

TEMPERATURE

There is a considerable amount of confusion with regard to temperature corrections. Biilmann and Krarup (5) found that the potential of the quinhydrone electrode, in a 0.1 *N* hydrochloric acid, against the hydrogen electrode, followed the equation:

$$\pi_t = 0.7175 - 0.00074t \text{ between } 0° \text{ and } 37°\text{C.}$$

The calculated and determined results fell within 0.4 of a millivolt. This equation, however, does not take into account the temperature coefficient of a calomel half cell, and where one of these is used, it is not correct to state that "the temperature factor as determined in Biilmann's investigations is 0.77 millivolts per degree deviation from 25°C."

The temperature coefficient of the whole cell varies with the half cell used. Biilmann and Tovborg-Jensen (6) have noted the temperature effects with Veibel's electrode. For the calomel cells corrections may be made as follows: Sørenson and Linderstrøm-Lang (26) give the temperature coefficients

0.1 *N* Cal. electrode—*N* Hyd. electrode	0.00006
1.0 *N* Cal. electrode—*N* Hyd. electrode	0.00024
3.5 *N* Cal. electrode—*N* Hyd. electrode	0.00039

Kolthoff and Furman (21, p. 141) give the saturated calomel electrode—*N* Hyd. electrode 0.00065. From these figures and the value 0.00074 above, the temperature coefficient for the quinhydrone electrode with the 0.1 *N* calomel electrode is 0.00006–0.00074 = −0.00068; with the *N* calomel electrode −0.00050; with the 3.5 *N* −0.00035; and with the saturated −0.00009.

From equation (F) at 18°C.:

$$\pi + 0.2864 = 0.0577 \log (\mathrm{H}^+) + 0.7044 \text{ for the quinhydrone}$$

compared to the normal calomel electrode

$$\text{or pH} \quad \frac{0.4180 - \pi}{0.0577}$$

With the temperature coefficient −0.00050, as shown above, Kolthoff (20). gives the formula

$$\mathrm{pH} = \frac{0.4181 - 0.00050\,(t - 18) - \pi}{0.0577 + 0.0002\,(t - 18)} \qquad \text{(G)}$$

This is the formula used by Hissink (19). It can be checked for 25°C. by using formula (D) with the value 0.2848 (11, p. 456) added to π; the agreement is good.

For the saturated calomel and the normal hydrogen electrodes at 18° the value is 0.2506 (11, p. 456). Ewing (15) prefers 0.2504. In the same way as before at 18°C.:

$$\mathrm{pH} = \frac{0.4538 - 0.00009\,(t - 18) - \pi}{0.0577 + 0.0002\,(t - 18)} \qquad \text{(H)}$$

Calculated from this at 25°, $\mathrm{pH} = \dfrac{0.4532 - \pi}{0.0591}$.

Equation (D) is used to check this, with the value 0.2464 (11) for the saturated calomel electrode. At 25°,

$$\mathrm{pH} = \frac{(0.6990 - 0.2464) - \pi}{0.0591} = \frac{0.4526 - \pi}{0.0591} \qquad \text{(I)}$$

This is fair agreement: the pH values differ only from 0.01 to 0.02.

From equation (I) a table can be made for the pH at 25°, or Baver's table (1) can be used. Baver's figures are slightly higher than those from equation

(I), but agree within 0.04 pH. By the use of figure 1 the amount of change in the pH for temperatures between 20° and 30° can be obtained directly for any given π. Corrections are *added* to the calculated pH if the temperature is *below* 25°, and subtracted if the temperature is higher. For example, if π is + 0.0313, from equation (I) the pH is 7.13 (Baver's table gives 7.15); but if the temperature of the experiment was 29°C., the corrected pH is 7.03, as the graph gives a correction of 0.10 pH at 29°, when π = 0.313. It will be seen that the temperature corrections are more important on the alkaline side. The following formula may also be used instead of the tables and graph:

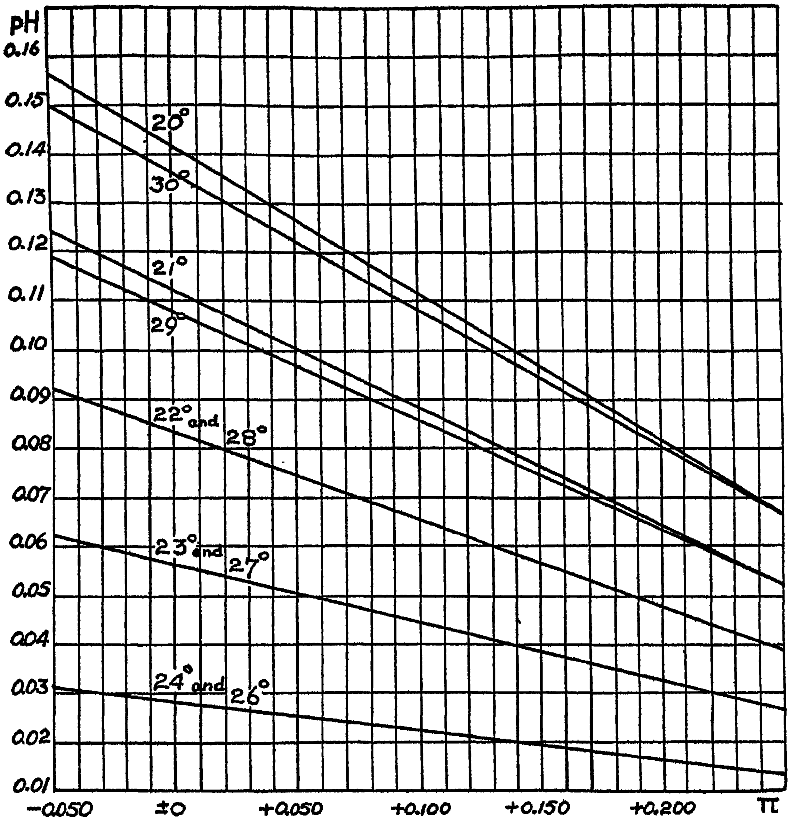

FIG. 1. pH CORRECTIONS AT TEMPERATURES OF 20° TO 30°C. FOR VARYING VALUES OF π

$$\text{pH} = \frac{0.4526 - 0.00009\,(t - 25) - \pi}{0.0591 + 0.0002\,(t - 25)}. \qquad \text{(J)}$$

Dawson (14) by taking the average value per degree (0.0006) of the Michaelis figures (11, p. 200), obtains 0.00014 for the temperature coefficient of the quinhydrone and the saturated calomel electrodes. Kolthoff and Furman (21) give the value 0.00065 per degree, which give 0.00009 for the coefficient. This seems to be more nearly correct, as the value is 0.00085 for the normal hydrogen electrode (26) and 0.00020 for the saturated calomel electrode (16, 15); this, however, would make only a small alteration in Dawson's table.

EXPERIMENTAL

In the experiments following, the quinhydrone electrode was prepared as described by Biilmann (3). The saturated KCl calomel half cell was used as the standard electrode. Connection was made by an agar bridge dipping into a closed solution of saturated KCl, in order to prevent diffusion and to keep down junction potentials.

The use of the KCl bridge with 5 per cent agar as suggested by Biilmann and Tovborg-Jensen (6) was found more satisfactory and caused less "drifting" of readings than bridges with a smaller percentage. Five grams of agar were placed in 100 cc. of the saturated KCl and the whole was weighed. The mixture was then boiled until the agar dissolved (heating below boiling produced very slow solution) and made up to the original weight with water. This prevented crystallization on cooling. It was found better to bring the solution almost to the solidification temperature before filling the tube by suction, in order to prevent contraction and the consequent drawing away from the sides of the tube. This contraction was further hindered by etching the inside of the glass tube with hydrofluoric acid containing a small amount of ammonium chloride, especially where much strain was put on the bridge, e.g., when it was being pushed into a thick soil suspension.

The electrode used for the soil suspensions with the quinhydrone was of flattened platinum wire. This was rather more durable than the usual flag when thrust into settled soil, particularly in the case of sand, and was much more convenient where stirring by motor was used to keep the soil in suspension.

Three types of soil were compared: a sandy soil, 13A, from the Miami series; a loam, 25A, from the Carrington; and a slightly heavier loam, 40A, from the low-lying soils of the Wabash series. Conductivity water (the laboratory distilled water redistilled over alkaline permanganate) was used in all cases.

The problems relating to the pH of a soil obtained by means of the quinhydrone electrode have been divided into two classes: first, preparation of the soil, e.g., soil water ratio, shaking, equilibrium; and second, measurement of the pH. The ratio of soil to water has not yet been standardized, some investigators have used 1:5 as with the hydrogen electrode (8), but there are reports (1) to show that a ratio of 1 soil to 5 water with the hydrogen electrode gives a pH value very close to the figures obtained with the quinhydrone electrode when the ratio is 1:1. A uniform method would prove valuable even though it had to be altered for special cases, e.g., for peat soils which would not give a suspension with an equal weight of water.

Biilmann and Tovborg-Jensen point out that an equilibrium between the soil and the water is desired, and that this is attained most rapidly if small amounts of water are used, they therefore advise a 1:1 ratio and recommend that the electrode should not be placed in the supernatant liquid, but lowered until entirely surrounded by soil, because the water nearest to the soil should come to equilibrium most quickly. They also suggest that if the substances causing the pH are more or less insoluble, there will be a saturated solution of these at both 1:1 and 1:5 ratios; but if they are fairly soluble, the solution at 1:1 may be much more concentrated than at 1:5. The pH would be altered by the increase of water with the more soluble but not with the comparatively insoluble substances.

Biilmann and Tovborg-Jensen further draw attention to the fact that the quinhydrone attains equilibrium instantaneously, so that if successive measurements show "drift," then either equilibrium between soil and water is not attained, or absorption of a component of the quinhydrone has taken place. In the second case a measurement immediately after mixing would be more nearly correct. In their experiments they found that the paste quickly attained the final value, and that usually no drift occurred at dilutions of 1:1 up to 1:10. They concluded that after shaking for a few seconds and measuring with the electrode submerged, the equilibrium reaction is measured. The method that was recommended, therefore, is to shake for a few seconds with a ratio of 1:1, stand half a minute for the soil to settle, and measure the pH with the electrode in the settled soil.

TABLE 1

Equilibrium reaction of three soils

TIME AFTER SHAKING	SOIL 40A		SOIL 25A		SOIL 13A	
	½ minute	10 minutes	½ minute	10 minutes	½ minute	10 minutes
ratio	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
1:1	5.74	5.71	5.57	5.57	5.89	6.23
1:5	5.97	5.95	5.86	5.75	6.26	6.36

This method was checked by the writers in the three soils mentioned, and the results are shown in table 1. Each figure is the average of three measurements which agree closely.

Soils 40A and 25A show no drift at 1:1 ratio. The soil should then be at equilibrium with the water in the paste, and no adsorption is indicated. The sandy soil, 13A, showed drift at both 1:1 and 1:5 ratios, indicating either no equilibrium or adsorption of one of the compounds of the quinhydrone. In all cases, with an increase of water, there is a change of pH, pointing to soluble substances which affect the acidity. If this suggestion is correct, increasing dilution should give a continual change of pH in the same direction, up to a maximum or minimum, when the pH would move towards the pH of the water used. Hissink and van der Spek (19) show very clearly that this turning point is reached in some soils.

EQUILIBRIUM SOIL:WATER

In order to test this equilibrium between soil and water, the three soils 40A, 25A, and 13A were treated as follows:

1. Shaking a few seconds, settling ½ minute and 10 minutes—electrode in paste.
2. Stirring with motor. A small stirrer at the bottom of the wide-mouth test tube, arranged to give a slight upward throw. Measurement taken while stirring; electrode in suspension.

TABLE 2

Results of test of equilibrium between soil and water, on three soils

	RATIO 1:1			RATIO 1:5		
	40A	25A	13A	40A	25A	13A
	pH	*pH*	*pH*	*pH*	*pH*	*pH*
1. Shake 2–3 seconds stand ½ minute	5.74	5.57	5.89	5.95	5.86	6.26
Shake 2–3 seconds stand 10 minutes	5.71	5.57	6.23	5.95	5.75	6.36
2. Stir with motor—just to mix	5.72	5.54	5.95	6.00	5.67	6.07
Stir with motor—½ hour	....	....	....	6.13	5.72	....
Stir with motor—3 hours	....	....	....	6.16	6.22	....
3. Rotating 2 hours	5.81	5.86	6.22	6.23	6.82	....
Rotating 6 hours	5.84	5.90	6.15	....	....	....
Rotating 20 hours	6.06	6.00	6.13	....	....	....
Rotating 24 hours	....	....	....	6.26	6.82	6.56
Rotating 36 hours	6.12	5.96	....	....	....	....
4. Colloid Mill 0.07 inch	....	....	....	7.42	*	7.03
0.014 inch	....	....	....	....	....	7.23
0.002 inch	....	....	....	....	....	7.39

* Alkaline.

3. Rotating machine. Soil and water rotated over and over, with speed slow enough to let mixture fall from one end of container to the other. Settle—electrode in paste.
4. Colloid Mill. The soil-water mixture passed through a colloid mill, cone type, with clearances from 0.07 to 0.002 inch. The particles did not settle out for a long time on standing—electrode in suspension.

The results show that there is no definite soil-water equilibrium in these soils, even in the 1:1 ratio. With 40A and 25A, which did not change when shaken a few seconds and allowed to stand 10 minutes, there is a progressive change toward the alkaline side on stirring or rotating, and a very decided rise in the pH value when sent through the colloid mill. The sandy soil, 13A, which showed a drift in the 10 minutes, did not change much by rotation at

1:1 ratio, but it also showed alkalinity through the colloid mill, and the finer the grinding the more alkaline the suspension became.

These figures are in accord with Fisher's suggestion (17) of a difference between the composition of the inside of the particle and the outside. Brown and Johnson (7) found indications of this when measuring the lime requirements of Iowa soils, while Cook (13), with New Jersey soils, obtained more acidity by grinding, indicating a more acid center of the soil particle.

FILTRATES AND SUSPENSIONS

Differences in the pH of the supernatant liquid, the paste and the suspension, emphasize the variations which may be expected in filtrates or centrifugates of soil suspensions. Carlton (9), working with 50 centrifuged extracts, found a difference in 16 soils ranging from 0.55 pH to 0.10. Similarly Gillespie and Hurst (18) found many differences when comparing the colorimetric pH of the centrifuged extracts with the suspensions electrometrically determined.

TABLE 3

Results of a comparison of filtrates with suspensions for various treatments of soils

SOIL	TREATMENT—RATIO 1:5	SUSPENSION	FILTRATES SUCCESSIVE 10-CC. PORTIONS					
			1	2	3	4	5	6
		pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
13A	Rotation 24 hours	6.56	6.95	7.13	6.93	6.98	6.89	....
25A	Rotation 24 hours	6.82	7.51	7.56	7.55	7.60	....	....
40A	Stir ½ hour	6.13	6.78	6.76	6.89	7.09	7.07	....
40A	Stir 3 hours	6.26	7.38	7.32	7.39	6.90	....	....
40A	Colloid Mill	7.42	5.88	6.67	7.05	6.83	6.78	7.22

Pierre (23), who prefers filtering to centrifuging, obtained fairly good agreement between the pH of the soil suspension and the filtered extract. He used a method of filtration with suction and washed filter papers, designed by Truog, and he considers that the differences usually found may be due to the effect of the CO_2 in the air on the little buffered filtrate. Sharp and Hoagland (24) with a CO_2 content of 10 per cent introduced into the electrode vessel, found small effect on any except alkali soils.

The larger part of these comparisons have been with colorimetric determinations and the hydrogen electrode. They are open to a number of objections mentioned by Pierre (23), but with the quinhydrone electrode the difficulties disappear. The figures in table 3 show some of the results of comparing filtrates with suspensions for various treatments of the soils. The Truog filtration method was used as described by Pierre. Each 10 cc. of the filtrate was tested for the pH; only a small amount of suction was used except on the last of the filtrate, when the soil was sucked dry.

The figures show that with these treatments there is a considerably larger difference between the filtrates and the suspensions than has been generally recorded. The variation in the successive portions of the filtrates is also marked.

In the soils used, the differences in the pH of the supernatant liquid and the paste became greater as the soil was made finer. If a soil-water mixture 1:5 is passed several times through the colloid mill and allowed to settle without stoppering, the suspension should be in equilibrium with air. A settling tube was prepared 9 feet long, with outlets every foot, so that samples at various heights could be drawn off. Table 4 shows a large increase in the pH value at the lower levels.

These differences can possibly be explained by the Donnan equilibrium—if two solutions are separated by a membrane, and there is a non-diffusible ion on one side, the concentration of the diffusible ion is greater on the side of the non-diffusible ion. No membrane is actually required, however, as long as an ionized part of the system is unable to diffuse. Comber (12) has pointed out that when a soil is treated with acid, certain non-diffusible (or slowly diffusible) ions, chiefly aluminum, come into solution. When the soil settles, these produce the Donnan equilibrium, the aluminum accumulates in the pore spaces and causes a lower concentration of the anion in the supernatant liquid. Similarly, soils treated with alkalis cause various slowly diffusible anions (e.g., silicates, aluminates) to be held in the pore spaces, and these produce lower cation concentration in the supernatant liquid. Hydrolysis of alkaline particles could cause the same phenomenon and would account for lower acidity in the paste.

TABLE 4

Soil pH values at different levels of settling tube

SOIL	13A	25A	40A
	pH	*pH*	*pH*
Suspension	7.39	Alkaline >8.5	7.42
Top of liquid	6.83	6.64	6.55
Liquid above paste	6.84	6.61	6.51
Top of paste	8.25	Alkaline >8.5	7.60
Middle of paste	Alkaline >8.5	Alkaline >8.5	7.75
Bottom of paste	Alkaline	Alkaline	7.59

If there is no equilibrium in the paste between the soil and the water, or at most a semi-stable equilibrium, the method adopted for the measurement of the pH must be empirical, but at the same time should give a reproducible value, characteristic of the particular soil. As there is a distinct difference in the pH values of the supenatant liquid and the paste, the mixture kept in suspension might be expected to be more representative of the soil. When the particles were brought into motion by stirring and the electrode was placed in the suspension, the readings either did not change from the simple shaking method (as in soil 40A, table 1) or more often the E.M.F. altered but failed to remain approximately constant. This did not hold out advantages over the electrode in the paste.

The stirring was applied in another manner, and one which prove more satisfactory. The soil sample was placed in the wide-mouth test tube and stirred for ½ minute; this mixed the water thoroughly with the soil without a grinding effect. The quinhydrone was then added to the mixture, which was shaken for a few seconds and allowed to stand for a half a minute as outlined. The electrode was placed in the paste. This method gave results varying slightly from the simple shaking and settling, but they were reproducible. Over 1000 measurements were taken on a number of soils with very different treatments, and in only a few cases the electrode failed to attain a comparatively constant E.M.F. In these measurements the agar bridge was placed near the electrode in the paste; there is sometimes a slight difference in the potential if the bridge is in the supernatant liquid.

SUMMARY

Further investigations have been made in the application of the quinhydrone electrode to the measurement of pH in soils.

1. Temperature corrections have been worked out for the quinhydrone electrode with the saturated KCl calomel half cell.
2. Biilmann and Tovborg-Jensen's method—mixing the soil and water by shaking for a few seconds—has been compared with stirring by motor, by rotary shaker, and by passing through a colloid mill, in order to investigate the equilibrium of the soil-water mixture. No definite equilibrium was found on the soils used.
3. By the use of a long settling tube, it was found that soil passed through the colloid mill increased the differences between the supernatant liquid and the settled soil. The Donnan equilibrium may account for the variation between the pH of the soil suspension, the supernatant liquid, and the paste. It is pointed out that there is still the question as to which of these should be measured for the soil reaction.
4. Reproducible values have been found by stirring the soil with water, using a 1:1 ratio, followed by the few seconds shaking and settling for a half minute, as described by Biilmann and Tovborg-Jensen.

REFERENCES

(1) Baver, L. D. 1926 The use of the quinhydrone electrode for measuring the hydrogen-ion concentration of soils. *Soil Sci.* 21: 167–179.

(2) Biilmann, E. 1921 Sur l'Hydrogénation des Quinhydrones. *Ann. Chim.* 15: 109–157.

(3) Biilmann, E. 1924 Oxidation and reduction potentials of organic compounds. *Trans. Faraday Soc.* 19: 676–691.

(4) Biilmann, E. 1924 On the measurement of hydrogen-ion concentration in soil by means of the quinhydrone electrode. *Jour. Agr. Sci.* 14: 232–239.

(5) Biilmann, E., and Krarup, I. 1924 The temperature coefficient of the quinhydrone electrode. *Jour. Chem. Soc.* 125: 1954–1956.

(6) BIILMANN, E., AND TOVBORG-JENSEN, S. 1927 On the determination of the reaction of soils by means of the quinhydrone electrode. *Trans. 2nd Comn. Internatl. Soc. Soil Sci.* B: 236–274.

(7) BROWN, P. E., AND JOHNSON, H. W. 1915 The effect of grinding the soil on its reaction as determined by the Veitch method. *Jour. Amer. Soc. Agron.* 7: 216–220.

(8) BURGESS, P. S. 1926 Report on reaction value of soils. *Jour. Assoc. Off. Agr. Chem.* 9: 138–141.

(9) CARLTON, E. A. 1923 A comparison of the Jones calcium acetate method for lime requirement with hydrogen-ion concentration of some Quebec soils. *Soil Sci.* 16: 79–90.

(10) CHRISTENSEN, H. R., AND TOVBORG-JENSEN, S. 1924 Investigations of methods used for determining the soil reaction. *Internatl. Mitt. Bodenk.* 14: 1–25 (*Chem. Abs.* 1925: 369–370).

(11) CLARK, W. M. 1925 The Determination of Hydrogen Ions. Baltimore, Md.

(12) COMBER, N. M. 1926 The action of non-diffusible ions in soil phenomena. *Trans. 2nd Comn. Internatl. Soc. Soil Sci.* B: 43–45.

(13) COOK, R. C. 1916 Effect of grinding on the lime requirement of soils. *Soil Sci.* 1: 95–98.

(14) DAWSON, L. E. 1926 The pH with quinhydrone electrode. *Sugar* 28: 211–214; 262–264; 310–312; 369–370.

(15) EWING, W. W. 1925 The preparation of electrolytic mercurous chloride in saturated potassium chloride for use in the calomel electrode. *Jour. Amer. Chem. Soc.* 47: 301–305.

(16) FALES, H. A., AND MUDGE, W. A. 1920 A study of the saturated potassium chloride calomel cell. *Jour. Amer. Chem. Soc.* 42: 2434–2453.

(17) FISHER, E. A. 1921 Studies of soil reactions: II. The colorimetric determination of the hydrogen-ion concentration in soils and aqueous soil extracts. *Jour. Agr. Sci.* 11: 45–65.

(18) GILLESPIE, L. J., AND HURST, L. A. 1918 Hydrogen-ion concentration—soil type—common potato scab. *Soil Sci.* 6: 219–236.

(19) HISSINK, D. J., AND VAN DER SPEK, J. 1926 Die pH Bestimmung des Bodens nach der Biilmann'schen Chinhydron-methode. *Trans. 2nd Comn. Internatl. Soc. Soil Sci.* B: 29–40.

(20) KOLTHOFF, J. M. 1925 Die Zuverlassigkeit der Chinhydron-elektrode für die Messung der Wasserstoffionenkonzentration in verschiedenen Lösungen. *Ztschr. Physiol. Chem.* 144: 259–271.

(21) KOLTHOFF, J. M., AND FURMAN, N. H. 1926 Potentiometric Titrations. New York.

(22) LAMER, V. K., AND PARSONS, T. R. 1923 The application of the quinhydrone electrode to electrometric acid-base titrations in the presence of air, and the factors limiting its use in alkaline solution. *Jour. Biol. Chem.* 57: 613–31.

(23) PIERRE, W. H. 1925 The H-ion concentration of soils as affected by carbonic acid and the soil-water ratio, and the nature of soil acidity as revealed by these studies. *Soil Sci.* 20: 285–305.

(24) SHARP, L. T., AND HOAGLAND, D. R. 1916 Acidity and adsorption in soils as measured by the hydrogen electrode. *Jour. Agr. Res.* 7: 123–145.

(25) SØRENSEN, S. P. L. 1909 Études Enzymatiques. II Sur la mesure et l'importance de la concentration des ions hydrogène dans les réactions enzymatiques. *Compt. Rend. Lab. Carlsberg* 8: 1–168.

(26) SØRENSEN, S. P. L., AND LINDERSTRØM-LANG, K. 1924 On the determination and value of π_0 in electrometric measurements of hydrogen ion concentrations. *Compt. Rend. Lab. Carlsberg* 15: (6) 1–40.

(27) VEIBEL, S. 1923 The quinhydrone electrode as a comparison electrode. *Jour. Chem. Soc.* 123: 2203–2207.

RETENTION OF PHOSPHORUS BY SOIL COLLOIDS[1]

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When a soluble phosphate such as monocalcium phosphate is added to a soil, it is well known that it is at once completely and permanently fixed. It is not appreciably affected by percolating water, and such solvents as nitric and citric acids will remove only relatively small percentages of the amount added. These facts have led to the assumption that phosphorus is held in the soil in one of two ways: first, it is retained as the insoluble phosphates of some of the soil bases, second, it is absorbed by the absorbing complex of the soil.

As early as 1878 Van Bemmelen (13) found that a soil, freed from exchangeable bases by leaching with acid, would no longer absorb phosphorus. Later, Rostworowski and Wiegner (10) removed all of the exchangeable divalent bases from a permutite by leaching with potassium chloride and found that the material lacked the power of retaining phosphorus. Russell and Prescott (11) studied the rate of extraction of phosphorus from soils, using nitric and citric acids, and then determined the rate of reabsorption. They found that the amount absorbed was proportional to the concentration of the anion. They also found that a number of other anions showed the same tendencies, if they were taken up at all. Comber (3) repeated some of the experiments of Russell and Prescott and found that, after the soil was leached with half normal hydrochloric acid until all of the calcium was removed, it would not take up the oxalate ion. He then concluded that the only way an anion could be removed from solution by a soil was by precipitation as an insoluble salt. He found, however, an appreciable absorption of phosphorus in nitric acid solution, even with this acid-washed clay. The absorption was greatest in the presence of 0.01 *N* nitric acid and less in either more acid or more basic solutions.

Gordon and his associates (5, 6, 12, 14) studied the absorptive properties of pure gels of alumina and iron oxide and the hydrosol and hydrogel of silica, and found that the absorption was greatest in the more acid regions. Silica absorbed very little, whereas the iron colloid took up as much as 380 mgm. of P_2O_5 per gram of colloid.

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[1] Part of a thesis submitted to the graduate school of the University of Missouri in partial fulfillment of the requirements for the degree of Master of Arts, August, 1927.

The author wishes to express his indebtedness to Dr. Richard Bradfield, of the department of soils, under whom this work was carried on, for his kindly supervision and criticism, and to Professor M. F. Miller, chairman of the department of soils, for critically reading the manuscript.

The facts derived from a study of the literature show that although there is a probable precipitation of insoluble phosphates in the soil, there is also good evidence of an absorption of phosphorus. The nature of the exchangeable bases and the hydrogen-ion concentration seem to be the important factors influencing the process, and it is probable that with a proper adjustment of these, both forms of retention may be illustrated.

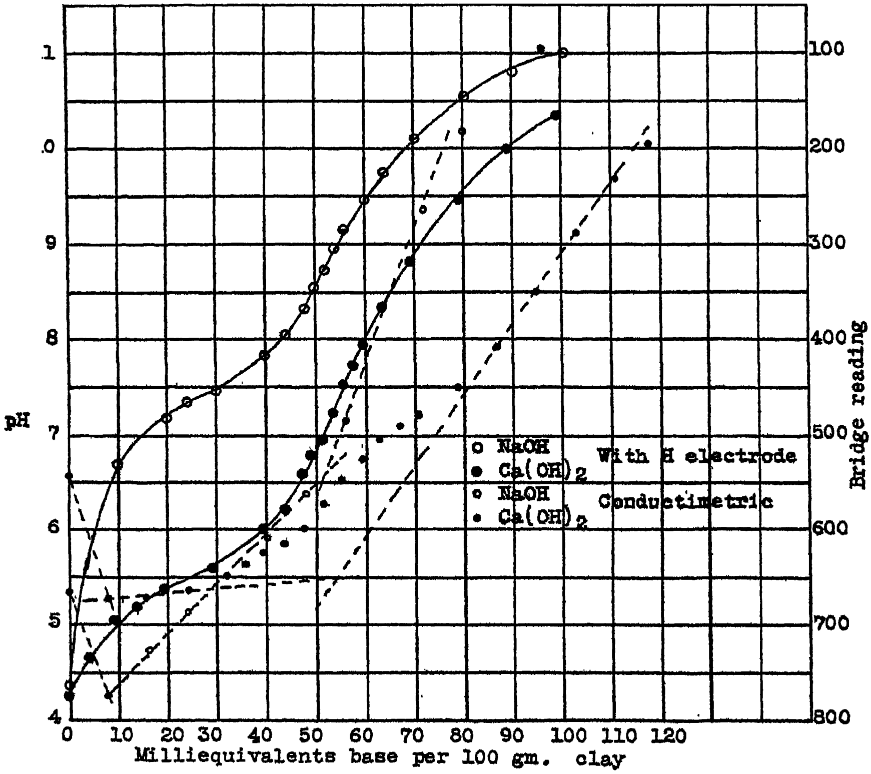

FIG. 1. TITRATION OF ELECTRODIALYZED COLLOIDAL CLAY WITH SODIUM AND CALCIUM HYDROXIDES

In this investigation the amount of phosphorus absorbed by electrodialyzed colloidal clay was determined. This clay is a very pure colloidal material from which the exchangeable bases have been removed by electrodialysis between parchment membranes and which in its final condition, contains hydrogen as its only exchangeable cation. It provides an excellent chance to study the absorption of phosphorus, because of the ease of regulation of the hydrogen-ion concentration, the exchangeable base, and the concentration of the anion—factors which heretofore may have caused many of the conflicting results.

EXPERIMENTAL

The material used in this research was supplied from a stock solution of the natural clay prepared from Putnam silt loam subsoil by the centrifugal method of Bradfield (1). It was electrodialyzed until free from exchangeable bases, resuspended, and standardized by means of a picnometer as well as by the oven-dry weight of a measured sample. This solution was used in the experimental work.

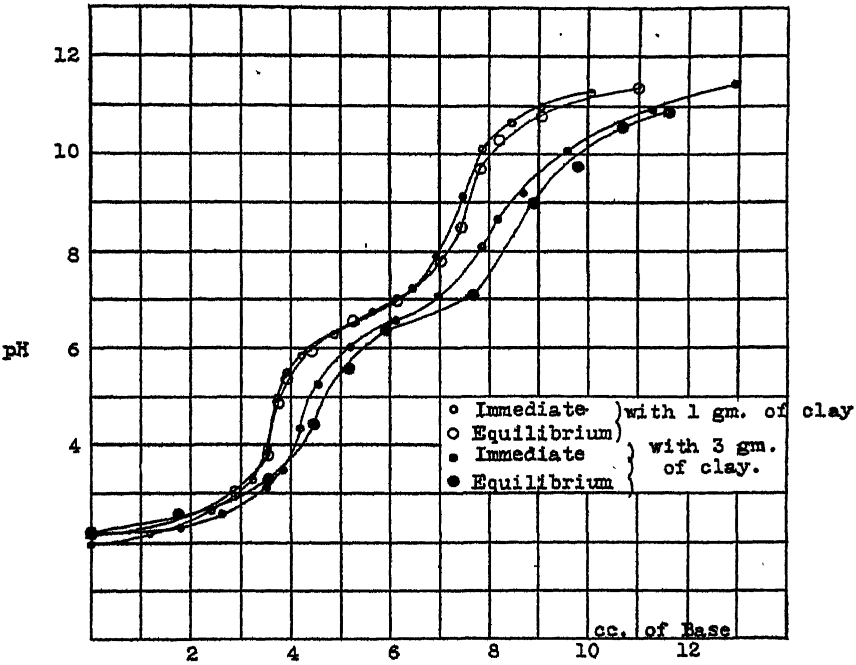

FIG. 2. TITRATION OF ELECTRODIALYZED CLAY + 10 CC. PHOSPHORIC ACID (0.9896 *N*) WITH SODIUM HYDROXIDE (0.2 *N*)

A number of titration curves were run to ascertain the approximate amounts of base to be added to produce definite pH values and also to give an indication of the rapidity of the reaction between the phosphorus and the clay. The curves of the clay alone are given in figure 1, and those of the clay mixed with phosphoric acid are given in figure 2. Using the values obtained from these curves, the experimental series were made up. The first was prepared as follows:

$$\left.\begin{array}{l}\text{50 cc. clay sol = 1 gm. oven-dry clay}\\ \text{50 cc. sodium chloride (1.0 } N\text{)}\\ \text{10 cc. phosphoric acid = 101.9 mgm. P}\\ \text{Sodium hydroxide (0.1780 } N\text{) and water}\end{array}\right\} = \text{250 cc.}$$

The sodium chloride was added as a flocculating agent. The whole was made to 250 cc. in a graduated flask, thoroughly mixed, and transferred to a

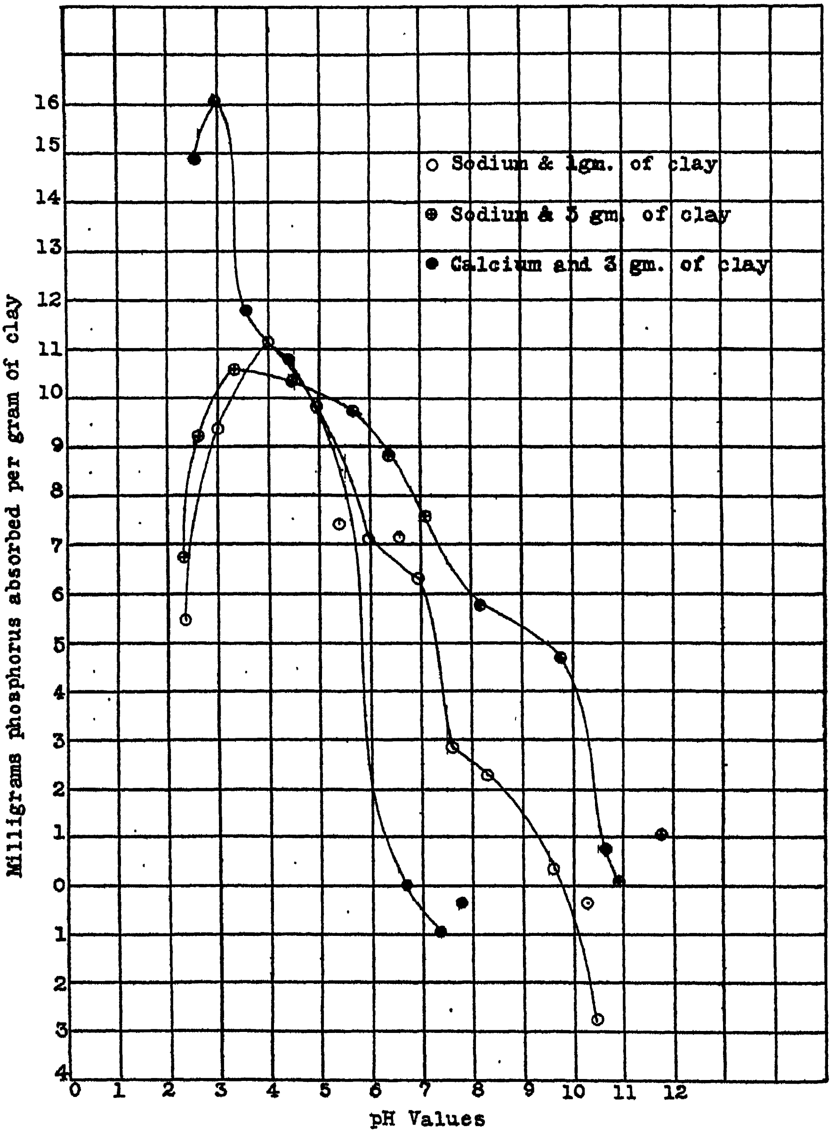

FIG. 3. ABSORPTION OF PHOSPHORUS BY ELECTRODIALYZED COLLOIDAL CLAY IN THE PRESENCE OF SODIUM AND CALCIUM

300-cc. erlenmeyer for storage. After standing 10 days with occasional shaking, portions of the supernatant liquid were removed for analysis. In case of necessity the samples were centrifuged to compact the clay, after which little difficulty was experienced in securing enough liquid for an aliquot. The pH determinations were made immediately afterward by the use of a hydrogen electrode that had been checked against a standard phthalate buffer. The phosphorus was determined according to the method of Jorgensen (7), after the silica had been removed by dehydrating with nitric acid, which also removed the organic matter. The curve of the absorption is given in figure 3.

This study revealed that:

1. The reaction between the phosphorus and the clay was practically instantaneous, as shown by the titration curves (fig. 2).
2. A curve drawn through the experimental points showed a maximum absorption at pH 3 to 4, with a rapid decrease on either side of this point and practically no absorption above pH 9.0.
3. The absorption by 1 gm. of clay was hardly enough to give conclusive results.

With these facts in mind, especially the latter, another series was made up which contained the following:

$$\left.\begin{array}{l}\text{97.8 cc. of clay sol} = \text{3 gm. of oven-dry clay}\\ \text{15 cc. sodium nitrate } (5.0\ N)\\ \text{10 cc. phosphoric acid} = \text{101.9 mgm. P}\\ \text{Sodium hydroxide } (0.1780\ N) \text{ and water}\end{array}\right\} = \text{250 cc.}$$

This series was handled similarly to the previous one except for a few minor details. The sodium nitrate was used as a flocculant instead of the sodium chloride to eliminate any error that the presence of the chloride ion might cause in the phosphorus determination. Two drops of toluol were added to prevent biological activity, after which the stoppers were securely inserted and coated with paraffin to insure against carbonate formation. The flasks were allowed to stand 5 days with occasional shaking, when aliquot portions were removed and the determinations made as usual. The absorption curve given in figure 3 shows marked coincidental tendencies with the first curve.

With the calcium series, a little different procedure was necessary. As only part of the phosphorus remained in solution above pH 5.5, it was necessary to ascertain the exact amounts present in solution at different pH values. This was done by running a series containing only calcium hydroxide and phosphoric acid. To keep the volume of the materials at a minimum a 0.2 normal solution of calcium hydroxide was prepared by dissolving calcium oxide in a 20 per cent sucrose solution. Then, because of the large amount of organic matter added in this solution, before the phosphorus determinations could be run, it was necessary to evaporate repeatedly with concentrated nitric acid to remove it. Usually three 50-cc. additions were sufficient to give a white residue. The curve showing the amount of the added phosphorus present in solution at the different pH values, is given in figure 4.

It was assumed that the presence of the clay had little effect on the solubility of the phosphate ion and that the difference between the amounts of phosphorus in solution with calcium alone and that found in the supernatant liquid over the clay would represent the amount absorbed. This is a safe assumption because in all cases there is a large excess of calcium present, allowing the phosphorus-calcium system to act as if alone, and thus probabl not changing the amount of soluble phosphorus present.

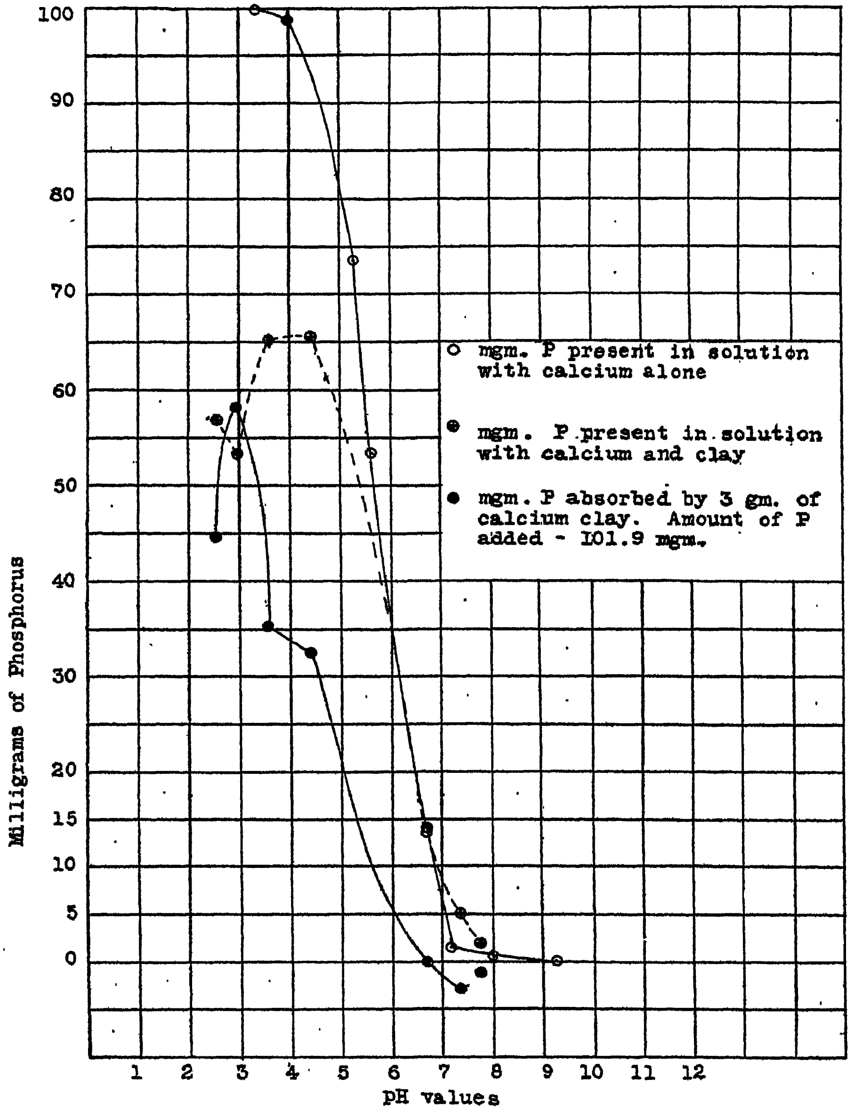

FIG. 4. AMOUNT OF PHOSPHORUS IN SOLUTION WITH CALCIUM, WITH CALCIUM AND CLAY AND THE ABSORPTION OF PHOSPHORUS BY CALCIUM CLAY

The calcium clay series was then made up as follows:

$$\left.\begin{array}{l}\text{97.8 cc. of clay sol} = \text{3 gm. of oven-dry clay}\\ \text{10 cc. phosphoric acid} = \text{101.9 mgm. P}\\ \text{Calcium hydroxide (0.1930 } N\text{) and water}\end{array}\right\} = \text{250 cc.}$$

This series was prepared and stored in a similar manner to those above except that it was allowed to stand 10 days instead of 5.

The three absorption curves given in figure 3 all show similar tendencies. The absorption by the calcium clay is about 50 per cent more than that by the sodium clay at the point of maximum absorption, but the difference between the sodium curves is slight. In all cases the absorption grades off sharply on either side of the maximum.

In the case of the sodium series there was a marked coloration of the supernatant liquid above pH 7.0, suggesting the presence of dissolved organic matter, which also produced a pronounced darkening of the clay itself. With the calcium series there was no such coloration, as the organic matter was insoluble in the calcium hydroxide. It was also observed that the volume of the flocculated clay was much less with the calcium than with the sodium.

DISCUSSION OF RESULTS

The data show that there is an appreciable absorption of phosphorus apart from that which may be held in the form of insoluble phosphates. The possibility of the formation of iron and aluminum phosphates is eliminated when it is remembered that where the maximum absorption occurs, the salts of these metals are practically all soluble, as shown by the work of Magistad (8). If the absorption were due to the precipitation of the phosphorus as phosphates of these metals, one would expect the absorption curve to be the inverse of the solubility curve of these phosphates. This is not true. The possibility of the formation of the phosphates of calcium is precluded because only the soluble portion of the added phosphorus was considered in calculating the amount of absorption.

When Comber (3) repeated the experiments of Russell and Prescott (11) he found the absorption to vary with the acidity in much the same manner as found in this investigation. Unfortunately, the pH values are not given, so the results are not strictly comparable, but one is led to believe that when the hydrogen-ion concentration is properly adjusted, phosphorus will be absorbed by soil whether calcium is present or not.

That the absorption of phosphorus may be shown to be a stoichiometric reaction in much the same way as base exchange seems to be altogether possible from the results of this study. The fact that the curves all rise and fall in the same regions, have maxima at the same hydrogen-ion concentrations, and show a general marked diminution of absorption at the higher pH values, seems to indicate that there is a specific reaction, the intensity of which is more markedly influenced by the cation and the hydrogen-ion concentration than by the concentration of the anion.

In figure 5 are given the absorption curves of phosphorus and sodium, the divergent nature of which seems to indicate that we are dealing with an amphoteric substance. This is believed to be a possibility in spite of the fact that Bradfield (1) found it impossible to reverse the charge on the clay particles by simply changing the hydrogen-ion concentration. It is possible that, although the major part of the clay is not amphoteric, it may contain a small percentage of some substance that is an ampholyte, and that this may be responsible for the absorption.

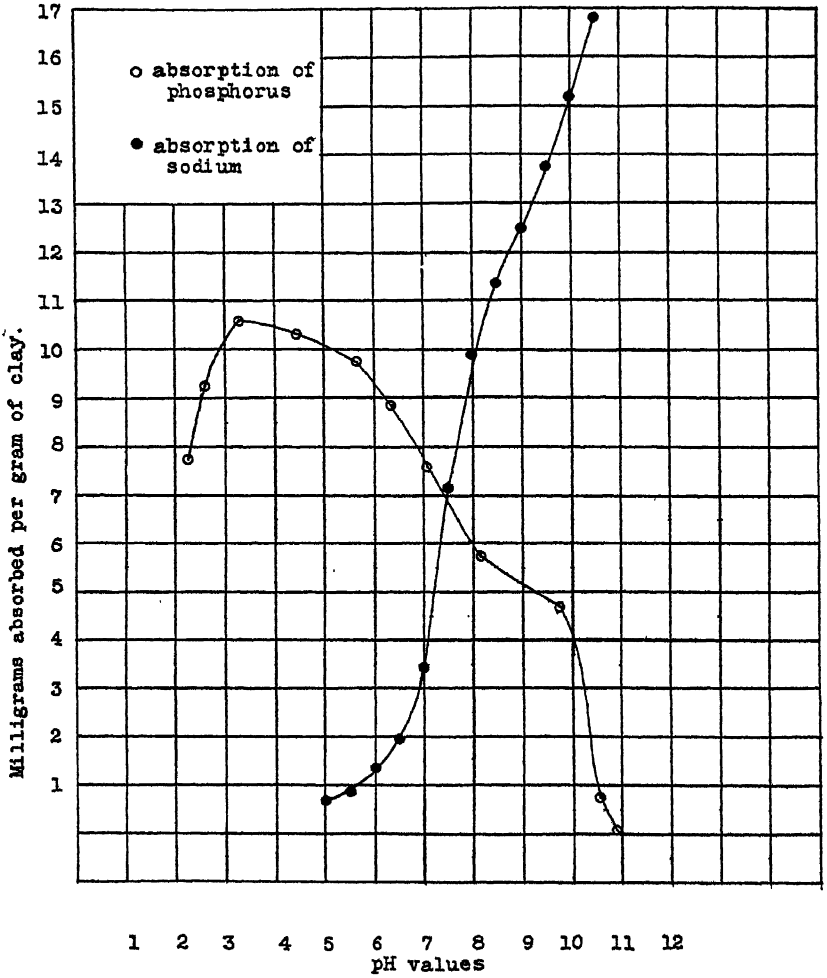

FIG. 5. CURVES SHOWING THE ABSORPTION OF PHOSPHORUS AND SODIUM BY ELECTRODIALYZED COLLOIDAL CLAY

It is possible that the organic matter may be the active constituent in this process, in view of the fact that it may show amphoteric properties. Peterson (9) has shown that as much as 50 per cent of the phosphorus of the soil may be liberated by treating the soil with hydrogen peroxide to oxidize the organic matter. If the organic fraction is responsible for some or all of the absorption, in the alkaline regions where it is soluble, the absorption should be less. The curve of the sodium clay (fig. 3) seems to bear this out.

SUMMARY

1. Electrodialyzed colloidal clay in the presence of calcium and sodium as cations, and at different pH values, absorbed a maximum of phosphorus at a pH of 3 to 4. The calcium clay absorbed one and one-half times the amount taken up by the sodium clay at this point. Above pH 9.0 there was no appreciable absorption.

2. The absorption per gram was practically the same whether one or three grams of sodium clay was present in the same concentration of phosphorus.

3. The maximum absorption occurred in the same region of pH where the iron and aluminum were most soluble, thus eliminating their responsibility in the absorption. It was not due to the formation of insoluble calcium salts because the soluble phosphorus only was considered.

4. The decrease in absorption by the sodium clay was accompanied by solution of the organic matter. This indicates the possibility of retention by the organic fraction.

REFERENCES

(1) Bradfield, R. 1923. The chemical nature of colloidal clay. Mo. Agr. Exp. Sta. Res. Bul. 60.

(2) Bradfield, R. 1923. The relation of hydrogen-ion concentration to the flocculation of colloidal clay. *Jour. Amer. Chem. Soc.* 55: 1243–1250.

(3) Comber, N. M. 1925. The rôle of electronegative ions in the reactions between soils and electrolytes. *Trans. Faraday Soc.* 20: 567–572.

(4) Cowan, E. S. 1925. Effect of hydrogen-ion concentration upon the absorption of calcium and exchange of bases by a colloidal clay. Thesis for A.M. degree, Univ. Mo.

(5) Gordon, N. E., et al. 1923. The adsorption and replacement of plant-foods in the colloidal oxides of iron and aluminum. *Soil Sci.* 15: 157–163.

(6) Gordon, N. E., and Starkey, E. B. 1922. The influence of soil colloids on the availability of salts. *Soil Sci.* 14: 1–7.

(7) Jörgensen, G. 1926. The determination of phosphoric acid as magnesium ammonium phosphate. *Analyst* 51: 61–72

(8) Magistad, O. C. 1925. The aluminum content of the soil solution and its relation to the soil reaction and plant growth. *Soil Sci.* 20: 181.

(9) Peterson, P. P. 1911. The effect of heat and oxidation on the phosphorus of the soil. Wis. Agr. Exp. Sta. Res. Bul. 19.

(10) Rostworowski, S., and Wiegner, G. 1912. Absorption of phosphoric acid through zeolites. *Jour. Landw.* 60: 223–235. (Abs. *Exp. Sta. Rec.* 28: 518; *Chem. Abs.* 6: 3304–3305.)

(11) RUSSELL, E. J., AND PRESCOTT, J. A. 1916. The reaction between dilute acids and the phosphorus compounds of the soil. *Jour. Agr. Sci.* 8: 65–123.

(12) STARKEY, E. B., AND GORDON, N. E. 1922. The influence of hydrogen-ion concentration on the adsorption of plant-food by soil colloids. *Soil Sci.* 14: 449–457.

(13) VAN BEMMELEN, J. M. 1878. Bas Absorptions vermögen der Ackererde. Landw. Vers. Sta., 23: 265.

(14) WILEY, R. C., AND GORDON, N. E. 1922. Adsorption of plant-food by colloidal silica. *Soil Sci.* 14: 441–448.

THE FATE OF FRACTIONAL INCORPORATIONS OF BURNT LIME IN TWO SOIL ZONES

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Rate per acre is of especial importance when either burnt or hydrated lime is used. Mooers (5) has recently reported the effect of a single moderate liming upon nitrogen economy during a 12-year period, and the further effect of a repetition, as measured by the changes induced during the succeeding 8 years, in a series of twenty $\frac{1}{40}$-acre plots. Slipher (6) has reviewed certain experiments which dealt with the response from light applications of lime, but no long-continued experiments are available to show the response to be expected from either single, or replicated, fractions of the usual 1-ton application.

Although economic increase in plant growth is the ultimate measure of the benefit from liming, it is important to determine both the initial and continuous effects resultant from different moderate additions. This paper presents such findings, from four rates of liming, as measured by the outgo of biological end-products and the conservation of the added alkali-earth, with the added factor of influence of zone of incorporation.

EXPERIMENTAL

The investigation covered the period between May 1, 1921, and May 1, 1925.[1] The successive annual precipitations were 51.7, 54.62, 45.56, and 39.71 inches. The fallow tanks comprised a group in a battery which has been described and illustrated as a whole (4). Details of treatment, and method of preparing and placing the soil were also given. Hydrated lime was incorporated at four rates—250, 500, 1000, and 2000 pounds of CaO per 2,000,000 pounds of soil, moisture-free basis, or $CaCO_3$-equivalent additions of 446, 893, 1785, and 3570 pounds, respectively. Previously reported plat studies with this fairly fertile brown loam (5) showed that the maximum of the four rates—2000 pounds of CaO—may be considered as an economic addition for certain crops.

Two tanks received lime at each rate. In one series the incorporations were made in the upper half, or zone, only, whereas in another series they were made solely in the lower zone. Periodic leachings from the full depth of soil were collected for both series. From previous related work with the same soil, it was known that practically complete absorption of the maximum incorporation occurred within 10 days (1). The soil may, therefore, be considered as having registered the increases in outgo due to 250 pounds, 500 pounds, 1000 pounds, and 2000 pounds CaO-equivalences of absorbed, i.e. non-carbonate, lime. In conformity, however, with corollary studies with limestone, dolomite, and magnesian limes, the calcium and magnesium results are reported both as pounds of $CaCO_3$ per 2,000,000 pounds of soil, and as per cent of addition.

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[1] The analyses of 1921–23 were made by Mr. H. Stanford, those of 1923–24 by Mr. T. D. Harden, and those of 1924–25 by Dr. R. M. Barnette. Each of these gentlemen was a sometime fellowship assistant, through provision made by the National Lime Association.

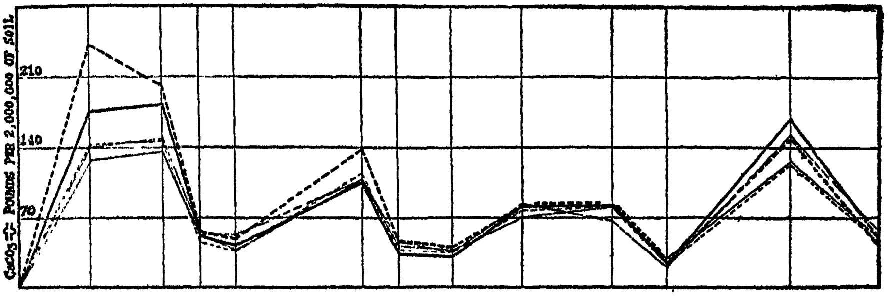
TABLE 1

Total calcium, magnesium, and calcium-magnesium leached during a 4-year period from 250-, 500-, 1000-, and 2000-pound CaO incorporations in the surface zone and subsurface zone of a loam soil—terms $CaCO_3$ equivalence per 2,000,000 pounds moisture-free soil

CaO* additions	FROM SURFACE-ZONE INCORPORATIONS							FROM SUBSURFACE-ZONE INCORPORATIONS						
	First year	Second year	Third year	Fourth year	Total for 4-year period	4-year increase in outgo over controls		First year	Second year	Third year	Fourth year	Total for 4-year period	4-year increase in outgo over controls	
						Actual	On basis of addition						Actual	On basis of addition
lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*
	Calcium as $CaCO_3$ ≎													
Controls	357	188	177	176	898	....	...	357	188	177	176	898	.....	....
250 (446)	373	189	181	171	914	16	3.6	378	193	184	174	929	31	7.0
500 (893)	394	184	175	207	960	62	6.9	444	182	171	178	975	77	8.6
1,000 (1,785)	452	169	197	212	1,030	132	7.4	572	231	217	181	1,201	303	17.0
2,000 (3,570)	543	220	188	190	1,141	243	6.8	1,064	438	314	265	2,081	1,183	33.1
	Magnesium as $CaCO_3$ ≎													
Controls	111	74	77	74	336	....	...	111	74	77	74	336	.....	....
250 (446)	109	74	67	69	319	−17	...	111	77	69	78	335	−1	....
500 (893)	116	74	59	76	325	−11	...	107	76	61	73	317	−19	....
1,000 (1,785)	123	74	68	74	339	3	...	109	76	54	62	301	−35	....
2,000 (3,570)	152	80	65	67	364	28	...	96	78	58	69	301	−35	....
	Calcium-magnesium as $CaCO_3$ ≎													
Controls	468	262	254	250	1,234	....	...	468	262	254	250	1,234	.....	....
250 (446)	482	263	248	240	1,233	−1	...	489	270	253	252	1,264	30	6.7
500 (893)	510	258	234	283	1,285	51	5.7	551	258	232	251	1,292	58	6.5
1,000 (1,785)	575	243	265	286	1,369	135	7.6	681	307	271	243	1,502	268	15.0
2,000 (3,570)	695	300	253	257	1,505	271	7.6	1,160	516	372	334	2,382	1,148	32.1

* Incorporated as $Ca(OH)_2$—$CaCO_3$ equivalence given in brackets.

The original nitrogen content of the soil was 0.1050 per cent. Its original total sulfur content of 0.0537 per cent was supplemented by a rainfall increment of 180.4 pounds of sulfate sulfur (3) during the 4-year period.


Surface-zone incorporations

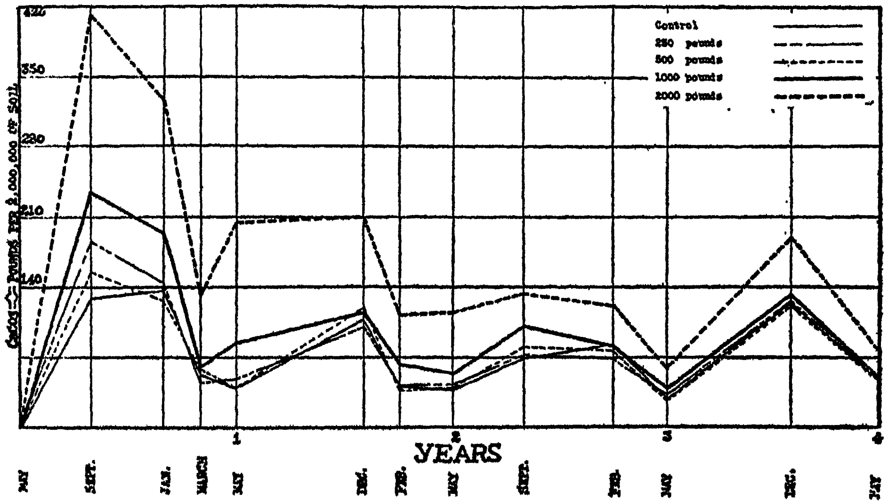

Subsurface-zone incorporations

FIG. 1. PERIODIC OUTGO OF CALCIUM DURING A 4-YEAR PERIOD FROM UNTREATED SOIL AND SOIL WITH ADDITIONS OF HYDRATED LIME AT THE RATE OF 250, 500, 1000, AND 2000 POUNDS OF CaO PER 2,000,000 POUNDS OF SOIL

CALCIUM AND MAGNESIUM OUTGO

From surface-zone incorporation

The annual and total losses of calcium and magnesium from the upper-zone incorporations are given in table 1 and the seasonal outgo in the curves of figure 1-A.

After the second leaching of the first year, and until the end of the 4-year period, the periodic losses of calcium from the untreated soil were practically the same as those from each of the three fractional additions to the surface zone. The several differences between the total calcium outgo from the control and the respective totals from the 250-, 500-, and 1000-pound CaO incorporations were, therefore, small for the 4-year period. The total enhancements in the outgo over the 898-pound loss from the control were, in order of magnitude of treatment, only 16, 62, 132, and 243 pounds for the four additions; or losses 3.6, 6.9, 7.4, and 6.8 per cent, respectively. These amounts, however, do not register the absolute releases of calcium to the leachings from the upper zone of treatment, since some stoppage was effected by the lower untreated zone. The periodic outgo of calcium (fig. 1) shows that there were accumulations of soluble salts during the warm months of minimum leaching rainfall. These accumulations were later leached by the fall and early winter rains. It is also apparent that only small amounts of soluble salts were produced during each of the four winter-months and that the outgo from each tank was generally very small at the May collections.

The magnesium losses from the control and those from the several treatments were in close agreement. Variations of such magnitude as to register against the control in the graph (fig. 1-A) would show only for the 2000-pound CaO addition, and then only for the first two periods of the first year. During that time the concentration of neutral calcium salts in the leachings from the heaviest upper-zone incorporations appeared sufficient to bring about some release of magnesium in the lower, untreated, zone—an effect which was precluded within the upper zone because of the protective action of the added lime.

From subsurface-zone incorporation

The losses of calcium and of magnesium from the incorporations in the lower zone are also given in table 1, and the seasonal outgo in figure 1-B. The periodic losses of calcium from the three fractional additions were practically the same as those from the control after the May to January period of the first year. The losses of 31 pounds, 77 pounds, and 303 pounds from these 446-, 893- and 1785-pound $CaCO_3$-equivalences, respectively, indicate the stability of the absorption complexes derived from the three well-disseminated fractional additions, in the absence of plant growth. When the 4-year average of these three enhancements in outgo is compared with a similar average for the initial year, it appears that 78.8 per cent of the total losses occurred during the first year. The maximum incorporation of 3570 pounds $CaCO_3$-equivalent (2000 pounds CaO) gave, however, an outgo in excess of that from the control for each of the 13 collection periods, and its total enhancement for the 4-year period was 1183 pounds, or 33.1 per cent of the amount incorporated. Even in this case of maximum outgo, the increase during the first year was 65.8 per cent of that for the full 4-year period.

Only the heaviest incorporation indicated any repression in the solubility of native magnesium within the zone of treatment. Since, with this exception, the magnesium leachings were again so concordant, the results were not graphed. The cumulative effect of each 4-year total shows, however, the same "reciprocal repression" that was noted in previous studies (2).

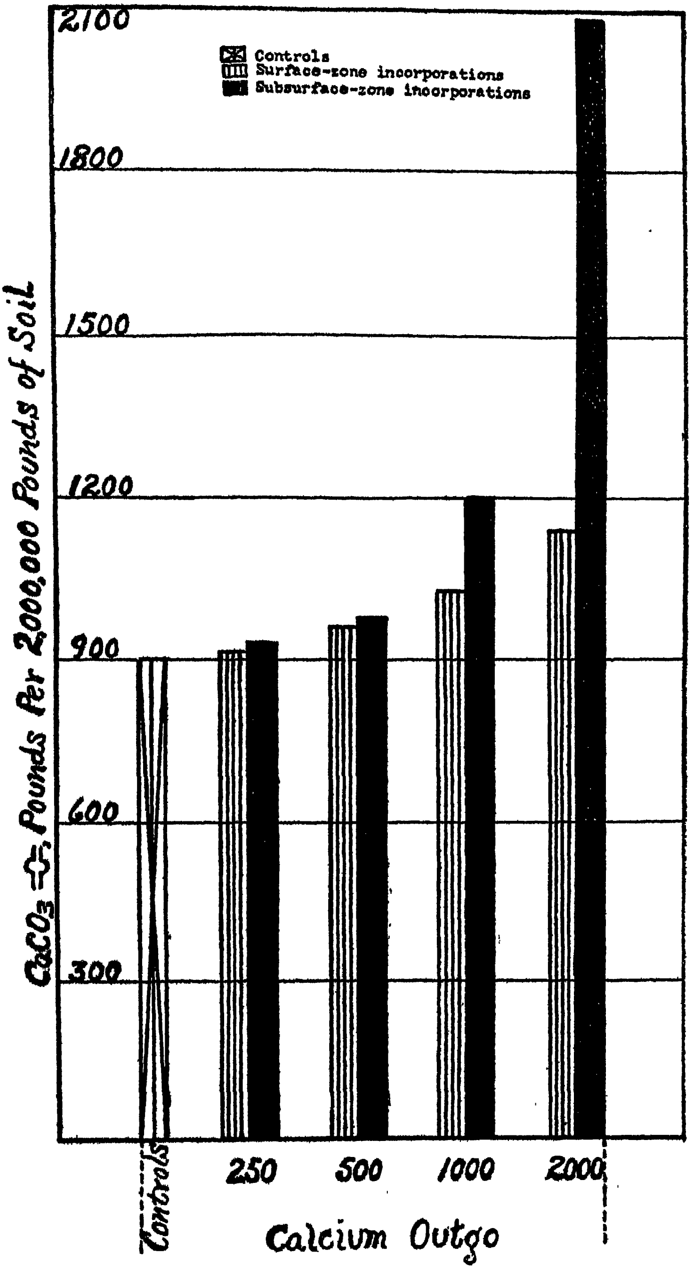


FIG. 2. TOTAL CALCIUM LEACHED DURING 4 YEARS FROM SURFACE-ZONE AND SUBSURFACE-ZONE INCORPORATIONS OF HYDRATED LIME AT THE RATES OF 250, 500, 1000, AND 2000 POUNDS OF CaO PER 2,000,000 POUNDS OF SOIL—TERMS OF $CaCO_3$-EQUIVALENCE POUNDS PER 2,000,000 OF SOIL

As influenced by zone of incorporation

The configurations of the curves for the two zones show considerable concordance in the calcium outgo from the three lighter treatments, after the first three leachings of the initial year. This is especially true of the 250- and 500-pound incorporations. The calcium supplied by these two smaller incorporations was insufficient to saturate the loam used, and the fixed calcium had acquired properties corresponding to those of the natural non-carbonate calcium content of the soil. But the two larger incorporations furnished more absorbed material to undergo hydrolysis and leaching. These activities were more extensive in the moist lower zone, and with no underlying untreated zone to diminish outgo through absorption, a greater release of calcium was obtained from the deeper incorporations. The loss from the 1000-pound CaO subsurface zone incorporation was 171 pounds in excess of that from the subsurface zone, and 70 per cent of that amount was accounted for during the first year. The lower-zone incorporation of 2000 pounds of CaO suffered a loss of 940 pounds $CaCO_3$-equivalence over that from the surface-zone incorporation, 521 pounds, or 55.4 per cent, of which occurred during the first year. Thus the loss from the subsurface-zone addition of 1000 pounds of CaO was actually more than that from the 2000-pound addition to the surface zone.

Practically all of the largest loss of calcium from the surface-zone incorporation may be assigned to the outgo of nitrates and sulfates. The $CaCO_3$-equivalence of the enhanced outgo of these two salts from the maximum surface incorporation is 250 pounds, as against the directly determined loss of 243 pounds. On the other hand, a similar comparison for the same addition to the subsurface shows that 93 per cent of the 1183-pound loss would be accounted for as calcium bicarbonate.

The magnesium losses induced by the two smaller incorporations in the subsurface-zone did not differ materially from those induced by the same incorporations in the upper zone. The two larger subsurface-zone incorporations, however, gave magnesium yields less than those from their corresponding surface-zone incorporations and also less than the magnesium outgo from the untreated soil, but none of the differences was extensive.

NITRATE OUTGO

The loss of nitrates is of prime importance in the maintenance of fertility. A considerable part of the calcium outgo is in this form. The important variables in the present experiment were rate of treatment and depth of incorporation. The factors of temperature ranges, fallow, and periodicity and amount of rainfall were constant. The meagre nitrate increment from rainfall may be disregarded in the present discussion.

The results from the surface-zone incorporations show that the fall and early winter rains were rich in nitrates and that only small amounts were leached during the winter and early spring months. This held for each of the

4 years. The hydrated lime incorporations failed to show any accelerative effect upon the generation of nitrates during the colder period of minimum bacterial activity. The 250- and 500-pound rates appeared to have induced very little increase in the nitrate outgo, even during the first year. The total nitrate loss from the 1000-pound treatment was twice that from the 2000-pound addition; the loss from the 1000-pound incorporation having been greater than that from the 2000-pound incorporation during 3 of the 4 yearly periods.

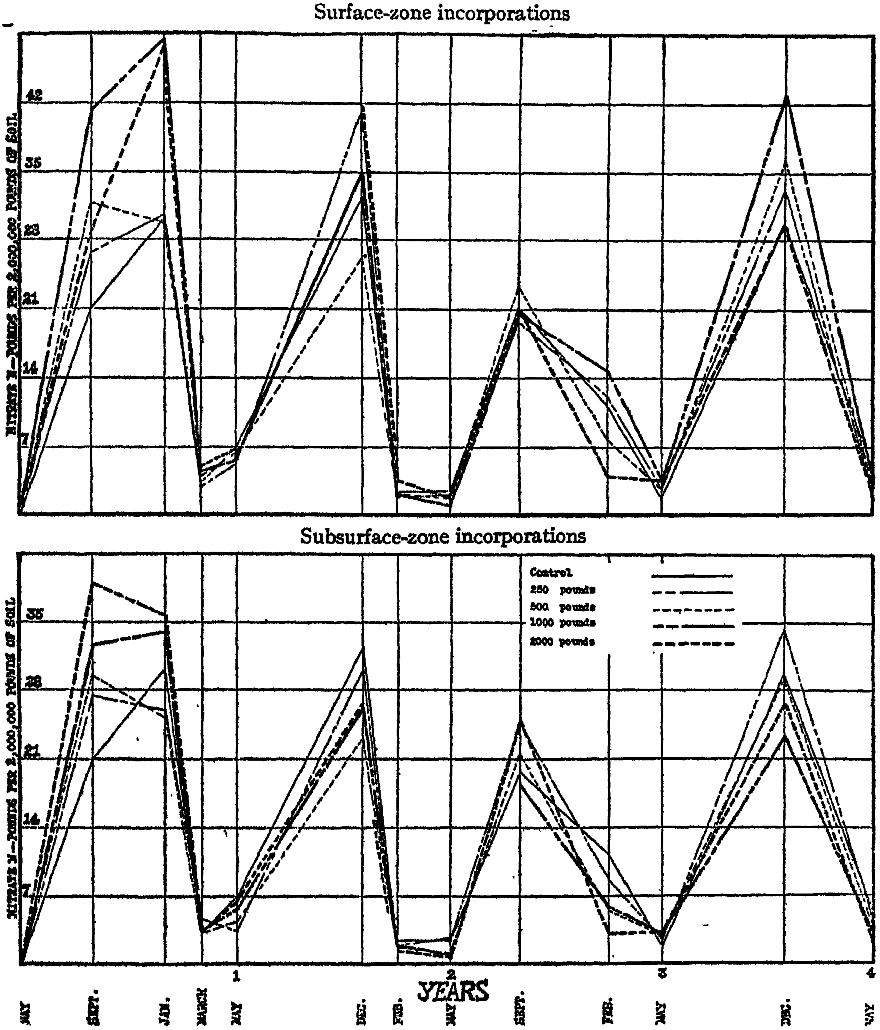


FIG. 3. PERIODIC OUTGO OF NITRATE NITROGEN DURING A 4-YEAR PERIOD FROM UNTREATED SOIL AND SOIL WITH ADDITIONS OF HYDRATED LIME AT THE RATE OF 250, 500, 1000, AND 2000 POUNDS OF CaO PER 2,000,000 POUNDS OF SOIL

In general, no enhancements in outgo came from the subsurface-zone incorporations, except during the first year, and then only from the two heavier incorporations. Small increases over the control showed as totals for the 4-year period in the case of the 250- and 2000-pound incorporations; whereas minus differences obtained from the 500- and 1000-pound incorporations.

The totals show that for each rate the nitrate outgo was greater from the surface-zone incorporations. There was only a small variation, however, in the case of the 250-pound incorporation. The maximum enhancement came from the 1000-pound incorporation, whereas the zone-effect increased induced by the 500- and 2000-pound incorporations were about equivalent.

TABLE 2

Nitrate nitrogen and sulfate sulfur leached during 4 years from 250-, 500-, 1000-, and 2000-pound CaO incorporations in the surface zone and subsurface zone of a loam soil—basis of 2,000,000 pounds moisture-free soil

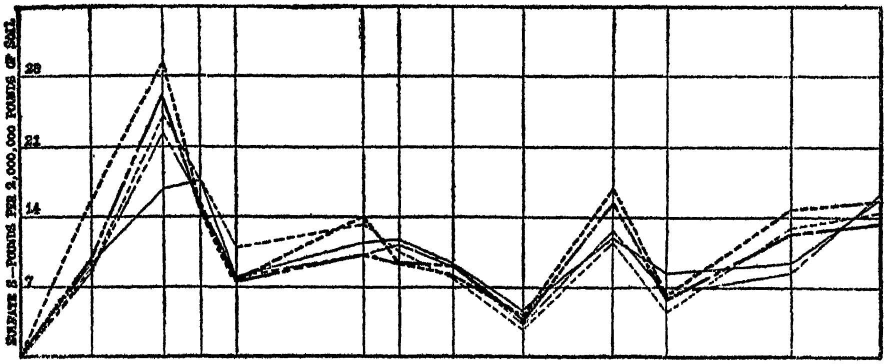
CaO* ADDITIONS	FROM SURFACE-ZONE INCORPORATIONS						FROM SUBSURFACE-ZONE INCORPORATIONS					
	First year	Second year	Third year	Fourth year	Total for 4-year period	4-year increase over controls	First year	Second year	Third year	Fourth year	Total for 4-year period	4-year increase over controls
lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
	Nitrate nitrogen											
Controls	63.1	37.1	34.1	34.7	169.0	.....	63.1	37.1	34.1	34.7	169.0	......
250 (446)	65.2	45.0	34.3	36.0	180.5	11.5	61.5	43.5	35.6	38.4	179.0	10.0
500 (893)	71.9	29.8	33.4	39.5	174.6	5.6	61.2	26.1	29.9	33.1	150.3	−18.7
1,000 (1,785)	100.0	38.2	38.8	46.9	223.9	54.9	75.8	28.9	27.5	25.8	158.0	−11.0
2,000 (3,570)	88.1	46.8	28.3	31.9	195.1	26.1	84.6	28.9	31.2	28.9	173.6	4.6
	Sulfate sulfur											
Controls	51.9	32.5	25.3	25.4	135.1	.....	51.9	32.5	25.3	25.4	135.1	......
250 (446)	54.7	30.3	22.3	24.7	132.0	−3.1	56.3	28.4	22.4	23.1	130.2	−4.9
500 (893)	61.5	31.8	18.8	27.4	139.5	4.4	59.4	28.4	23.3	27.2	138.3	3.2
1,000 (1,785)	58.5	28.6	24.8	24.9	136.8	1.7	58.9	26.6	23.6	31.3	140.4	5.3
2,000 (3,570)	67.2	31.3	27.1	30.2	155.8	20.7	67.2	27.1	25.1	29.1	148.5	13.4

* Incorporated as $Ca(OH)_2$—$CaCO_3$ equivalence given in brackets.

This particular loam has shown ability to fix lime in quantities considerably larger than the present maximum addition, but, although unsaturated with calcium, the pH values of its leachings were otfen in excess of 7, so that apparently it was not so acidic as to require extensive lime additions to promote nitrification. The remarkable similarity between the nitrate curves shows that, under fallow, the dominant factor in nitrate production was that of season, and that the lime incorporations were of secondary importance

In view of the uniformly small supplies of nitrate nitrogen present in the collections on the first day of each May, in a location where the season is well advanced at that period, it is easy to comprehend why top-dressings of readily available nitrogen are advantageous—certainly where the soil had not been recently turned.

Surface-zone incorporations



Subsurface-zone incorporations

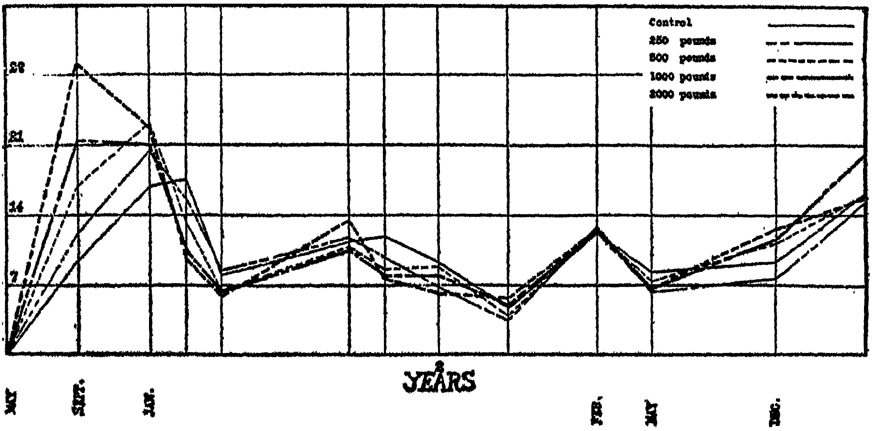


FIG. 4. PERIODIC OUTGO OF SULFATE SULFUR DURING A 4-YEAR PERIOD FROM UNTREATED SOIL AND SOIL WITH ADDITIONS OF HYDRATED LIME AT THE RATE OF 250, 500, 1000, AND 2000 POUNDS OF CaO PER 2,000,000 POUNDS OF SOIL

SULFATE OUTGO

The sulfate losses are given in table 2. Each rate caused some increase during the first year. The three lighter treatments yielded about equivalent amounts, and the maximum rate gave the greatest outgo. On the whole, no further acceleration was registered by any one of the four additions. The 4-year totals therefore showed relatively small increases in sulfate outgo for the three heavier incorporations, and none for the lightest treatment. The largest outgo from the 2000-pound incorporation was less, however, than the amount brought down by rain water, so that there was no actual loss of sulfates to be attributed to either enhanced sulfofication, or to a lessening of the soil's ability to hold sulfates. The greater leaching of sulfates during the winter months does not prove that spring and summer accumulations of sulfates were washed out by the late fall and early winter rains less readily than were the nitrates; nor does it follow that sulfate generation exceeded that of nitrates during the winter months, since the rainwaters carried more sulfates during the coal-burning season (3).

The annual and 4-year losses further show that small differences in sulfate outgo resulted from variation in depth of incorporation and the general concordance of the sulfate curves shows that losses of sulfates were influenced mainly by season and by rainfall increments.

CONSERVATION OF CALCIUM, AS RELATED TO RATE OF LIMING

During recent years it has been held that it is well to make small additions of lime each year. In support of this viewpoint, it is contended that the practice is economic, and that the cumulative effect is such as to insure the gradual but certain coming of legumes from light seedings in mixtures. In those soils, where optimum bacterial activities may result from the smaller incorporations, and where the factors of base unsaturation and nutrient requirements are not predominant, the progressive incorporation plan may possess distinct merit.

The present study throws some light upon the proportionate conservations of single-lime additions at different rates. The several additions did not so alter the content of leachings as to indicate any interchange of bases. They displaced hydrogen ions and were "fixed" as non-carbonate forms in the soil mass within a few days, hence the losses of calcium may be considered as measuring the release of calcium from the absorption complexes, through hydrolysis and the attack by bacteriologically-engendered acids. The soil containing the lighter treatments, especially, were far from being "saturated," and the enhancement in the alkali-earth outgo from these additions were practically negligible. The complexes derived from the lighter incorporations were less extensively disseminated and less extensively hydrolyzed by the soil water and therefore less extensively leached. The heavier treatments brought the soil to a condition more nearly approaching saturation—a condition permitting greater hydrolysis and consequently greater leaching. This, together with the factor of greater surface exposure to the action of the soil water, was responsible for a greater calcium outgo which could not be accounted for entirely by increases in soluble neutral salts engendered through bacterial action. Insofar as conservation of single additions of lime is concerned, the smaller incorporations gave proportionately the larger conservations. It does not follow, however, that the same would hold true if the 4-year outgo from a full single economic addition were compared with that obtained from the same amount of lime supplied through fractional additions annually. Here an intervening factor, that of "aging," comes into play. After a single addition has been made, the aging process begins its influence upon the full amount of absorbed material shortly after its incorporation. But with smaller additions, repeated so as to supply in the aggregate the same amount as that supplied in the single addition, the aging process is delayed until after the fixation of each successive fraction. This factor may vary decidedly, as influenced by the two methods of adding the same amount of lime to provide for soil needs over a given period of years. The repetitions may be considered as furnishing successive fresh streams of calcium to the soil solution. Very little is now known concerning the cumulative effect of such repetitions, as compared to heavier single additions. This problem is now being studied.

SUMMARY

The fate of completely fixed lime, added at four rates and in two zones, was studied by means of lysimeters over a 4-year period.

The losses of calcium from 250- and 500-pound CaO additions to both zones were practically the same as the loss from the untreated soil. The influence of zone of incorporation was especially marked for the 1000- and 2000-pound rates. The loss from a 1000-pound subsurface addition was greater than that from a 2000-pound addition to the surface zone. The losses of calcium were accounted for in the main by the outgo during the first year.

Magnesium losses were rather uniform, with evidence of repressed solubility in the zone where the lime additions were incorporated.

The increases in nitrate outgo were accounted for mainly during the first year. Nitrates accumulated during the summer months were removed by early winter rains. There was apparently no generation of nitrates thereafter until after the May collections of percolates.

The high concentration of sulfate leachings during the winter months was attributed to the greater precipitation of sulfate sulfur during that season.

The augmented losses of calcium from the lower-zone incorporations were shown to be due to bicarbonates derived from the fixed lime, whereas the losses from surface incorporations may be accounted for mainly by the outgo of biological end-products.

The relation of rate of liming to calcium conservation is discussed.

REFERENCES

(1) MacIntire, W. H. 1919 The carbonation of burnt lime in soils. *Soil Sci.* 7: 325.

(2) MacIntire, W. H. 1926 Reciprocal repression by calcic and magnesic additions in surface soil. *Jour. Amer. Soc. Agron.* 18: 482.

(3) MacIntire., W. H., and Young, J. B. 1923 Sulfur, calcium, magnesium, and potassium content and reaction of rainfall at different points in Tennessee. *Soil Sci.* 15: 207.

(4) MacIntire, W. H., and Shaw, W. M. 1925 The disintegration of limestone and dolomite separates as influenced by zone of incorporation. *Soil Sci.* 20: 417.

(5) Mooers, C. A. 1926 Effects of liming and green manuring on crop yields and on soil supplies of nitrogen and humus. Tenn. Agr. Exp. Sta. Bul. 135.

(6) Slipher, J. A. 1921 The economics of soil liming on appraisal of monetary returns. *Jour. Amer. Soc. Agron.* 17: 211.

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